ANALYSIS OF ANTHOCYANIN COLOUR STABILITY FROM FRUITS PULP OF *MELASTOMA MALABATHRICUM*

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DECLARATION

I hereby declare that the work reported in this thesis is my own unless specified and duly acknowledged by quotation.

(NORKASMANI AZIZ)

SEPTEMBER 2012

ABSTRACT

The aim of this dissertation is to evaluate suitability of anthocyanin from *M. malabathricum* as potential natural colourant for coating system and also to evaluate the colour stability of anthocyanin colorant with and without Ferulic acid (FA) stabilising agent in a polyvinyl alcohol (PVA) binder coating system. Besides that the purpose for this project is to analyse the colour stability of potential natural colourant in a coating system in terms of percentage of FA and pH toward UV-B irradiation using CIE system. The anthocyanin colourant from *M.malabathricum* using acidified methanol for crude and purified colourant. Purification was performed by liquid-liquid partition and ion exchange column chromatography. Different percentages of Ferulic acid as stabilising agent in order to improve resistance towards UV-B irradiation during the exposure period. FA added colourant was mixed with PVA to develop a coating system. To test colour stability of crude and purified anthocyanin colorant and anthocyanin-PVA blend towards UV-B irradiation,CIE colour analysis was carried out. CIE results were analysed in terms of L*C*H*a* and b* co-ordinate.Colour differences ΔE and saturation of colour, (s) were calculated in order to evaluate the visual colour variation in this study. CIE results shows that the colour variation of anthocyanin and anthocyanin-PVA blend both crude and purified affected by the addition of FA. The addition of 3% FA at pH3 showed better stability.

ABSTRAK

Tujuan disertasi ini adalah untuk mengkaji kesesuaian antosianin daripada *M. malabathricum* sebagai pewarna semulajadi yang berpotensi untuk sistem salutan dan juga untuk menguji kestabilan warna antosianin yang ditambah Ferulik asid(ejen penstabil) sebagai pewarna dalam alkohol polivinil (PVA) sistem salutan. Selain itu, tujuan projek ini adalah untuk menganalisis kestabilan warna pewarna semulajadi yang berpotensi dalam sistem lapisan dari segi peratusan FA dan pH terhadap sinaran UV-B menggunakan sistem CIE. Antosianin daripada *M.malabathricum* diekstak mengunakan metanol berasid. Penyucian telah dilakukan melalui proses pertukaran ion. Peratusan asid ferulik yang berbeza ditambah sebagai ejen untuk meningkatkan rintangan ke arah penyinaran UV-B. pewarna yang ditambah FA dicampur dengan PVA untuk menghasilkan sistem salutan. Untuk menguji kestabilan pewarna dan antosianin tulen, pewarna dan antosianin-PVA campuran di letak di bawah penyinaran UV-B, Analisis warna CIE telah dijalankan. Keputusan CIE dianalisis dari segi L* C* H* a* dan b* ko-ordinate. Warna perbezaan ΔE dan ketepuan warna, (s) telah dikira untuk menilai perubahan warna visual dalam kajian ini. Keputusan CIE menunjukkan bahawa perubahan warna antosianin dan antosianin-PVA dipengaruhi oleh penambahan FA. Penambahan FA 3%, pH3 menunjukkan kestabilan yang baik.

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List of Symbols and Abbreviations

FA	Ferulic acid
TFA	Trifluoroacetic acid
PVA	Poly (vinyl) alcohol
UV-B	ultra violet B

CHAPTER 1: INTRODUCTION

1.1. Background

The coatings industry is a mature industry that has been undergoing a continual change in technology. Most products require some type of coating such as paint, stain or sealer. The use of coatings is widespread, (Weisse, 1997). Coating is defined as mixtures of various materials of four groups materials namely resin, pigments, solvents and additives. The word coating describes the resulting dry continuous film obtained by the process, applying a material (usually liquid) on a substrate surface using prescribed methods. Coatings may be described by their appearance (clear, pigmented, metallic, glossy) and by their function (corrosion protection, abrasion protective, skid resistant and decoration) (Gutoff,2006)

Coatings are used in a wide range of stand-alone products .Coated products vary from painted automobiles to colour photographic films, etc. These coatings replace air on a substrate with a liquid to give the final product its desired properties. Research efforts have been intensified to produce coating products especially paint from various types of materials with different formulations.

Paint is a coating product widely used in peninsular Malaysia. Paint is used to protect, preserve, decorate or add functionality to an object or surface by covering it with a pigmented coating. An example of protection is the covering of metal to retard corrosion, and the painting of a house for its protection. Paint may be used to add functionality by modifying light reflection or heat radiation of a surface. Another example of functionality is the use of colour to identify hazards or to identify the function of equipment, such as pipelines or military ammunition (Gutoff, 2006).

1.2. Problem statement

Nowadays, synthetic paints are widely used because of their good performance and quality, but this type of paint can cause permanent injuries and death due to their toxicity (OCCAP)**.** Non-toxic or natural paints are mostly manufactured as alternative paints to overcome this issue. Customer demands for Total Quality Management (TQM) and IS0 certification have forced the paint industry to place a major emphasis on establishing quality control in their manufacturing processes (Weise, 1997) .The competitive environment of this industry provides the drive needed to develop coatings that utilize less expensive and safer raw materials (Gutoff, 2006).

Colour is the basic building element of coating. It is a mixture of pigment and binders. The composition gives the colour quality. The main aspects of this quality are colour stability and hiding power. Colour stability and hiding power can be strongly time dependent due to the interactions between chemical species within the mixture and with the environment (Hradil et al., 2003). According to Chou et al. (2007), The study of natural colorants is an intensive and active area of investigation due to the growing interest of substituting synthetic colorants with toxic effects in humans (Castaneda et al., 2009).

1.3. Objective of this study

The objectives of this study are.

1. To evaluate the suitability of anthocyanin from *M. malabathricum* as potential natural colourant for coating system
2. To evaluate the colour stability of anthocyanin colorant with and without Ferulic acid (FA) stabilising agent in a polyvinyl alcohol (PVA) binder coating system.
3. To analyse the colour stability of potential natural colourant in a coating system in terms of percentage of FA and pH toward UV-B irradiation by using CIE system.

1.4. Scope of study

Growing consumer demands for less toxic high performance product provides the drive needed to develop safe coating. Non-toxic and natural coatings can be made from natural ingredients like water, plant oils, plant dyes (natural colourant), and other ingredients. Therefore, natural colourants become an active and intensive study in order to fulfill the urge of consumer demand in substituting synthetic colourant. In order to accomplish this target, further studies on natural colourant from plants such as *M.malabthricum* were undertakes in this dissertation. Continuous study on *M.malabathricum* anthocyanin colourant is important in order to provide more information about suitability of this natural colourant as raw material for natural coating. This is because *M.malabathricum* from the family Melastomataceae an important source of natural anthocyanin colourant for coating that is environmental friendly and less expensive material (Wong, 2008). However, this natural colourants are less stable and easily degrade. Due to this reason, it is the intention of this study to increase the colour stability of *M.malabathricum* natural colourant in a coating system.

Chapter Two of this dissertation contains the literature review regarding the coating components and materials used in this study. Chapter Three presents the sample preparation methods and the techniques used to analyse the colour stability of the sample prepared. Chapter Four displays results of colour analysis and stability of the *M.malabathricum* coating system by using CIE system. Chapter Five displays resultsbof colour analysis system comprising Poly(vinyl alcohol), PVA. Chapter Six discusses the results obtained from the colour analysis study. Finaly, Chapter seven concludes the thesis with some suggestions for further works that may be useful to further improve the existing coating systems.

CHAPTER 2: LITERATURE REVIEW

2.1. Pigment and colourant

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Pigment contains a molecule-specific structure called chromophore that determined the colour produced. When the chromophore captures light energy, electrons are excited from low to high orbitals. The eyes capture the reflected or refracted unabsorbed energy and generate neural impulses that are transmitted to the brain where they are interpreted as a colour (Hari, 1994). Colourants can be categorized into natural and synthetic. Natural colourants are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic colourants are man-made (Bauernfeind et al., 1981)

2.1.1. Natural plant colourant

Plants produce more than 200 000 different types of compounds (Fiehn, 2002), including many coloured (pigmented) compounds which can be found in leaves, flowers and fruits. These compounds can be classified by their structural characteristics as follows: tetrapyrrole derivatives (chlorophylls and heme colours), isoprenoid derivatives (carotenoids), N-heterocyclic compounds different from tetrapyrroles (purines, pterins, flavins, phenazines, phenoxazines and betalains), quinones (benzoquinone, naphthoquinone, anthraquinone), melanin (Delgado-Vargas et al., 2000). benzopyran derivatives (anthocyanins and other flavonoid pigments).

2.2. Anthocyanin

Anthocyanins are a group of flavonoids that are major sources of colour in flowers and fruit that impart brilliant red and blue colors. For example, the colours of berry fruits, such as strawberry, bilberry and cranberry, are due to many different anthocyanins (Delgado-Vargas et al., 2000). Anthocyanins are complex and water-soluble molecules (Castaneda et.al., 2009) which containes phenolic substances and widely found in vascular plants. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening (Guisti and Wrolstad, 2003).

2.2.1. Basic structure of anthocyanin

Anthocyanins consist of aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s). They occur in nature as glycosides of anthocyanidins and may be acylated with aliphatic or aromatic acids (Guisti and Wrolstad, 2003). Aglycone or the flavyllium cation is the main part of anthocyanins,The flavylium cation contains conjugated double bonds responsible for absorption around 500 nm causing the pigments to appear red to the human eye. Aglycones or anthocyanidins has a C6-C3-C6 carbon skeleton basic structure (Brouilard, 1982). The number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of the attachment, and number of aliphatic or aromatic acids attached to sugars in the molecule relates to the differences between individual anthocyanin, figure 2.1. In other words, glycoslyation of hydroxyl groups, nature of glycosyl units, substitution patterns, and potential aliphatic and aromatic acylation indicate the type of anthocyanin (Andersen and Jordheim, 2006). These substitution patterns are responsible for the different color parameters (lightness,chromaticity and hue) observed for different anthocyanin pigments. The hydroxyl group at carbon **3'** is very significant in changing the color of anthocyanins from yellow-orange (e.g. strawberries, pelargonidin-based pigments) to bright red (e.g. blackberries, with more than 80% cyanidin 3-*O*- *β*-*D*-glucoside), and to the bluish red of young red wines (largely caused by malvidin 3-*O*-*β*-*D*-glucoside) (Schwarz and Winterhalter, 2003).

There are six common anthocyanidins in higher plants (a) pelargonidin (Pg),(b) peonidin (Pn), (c) cyanidin (Cy), (d) malvidin (Mv), (e) petunidin (Pt) and (f) delphinidin (Dp) which only differ by the hydroxylation and methoxylation pattern on their B-rings (figure 2.2). The glycosides of the three non-methylated anthocyanidins are the most widespread in nature, being present in 80% of pigmented leaves, 69% of fruits and 50% of flowers (Kong et.al, 2003). Anthocyanidins are very unstable, rarely found in fresh plant material and therefore occur mainly in glycosylated forms, where sugar substitution enhances the stability and solubility of anthocyanin molecule (Clifford, 2000; Giusti and Wrolstad, 2003). The most common sugar moieties glycosylating aglycones are glucose, galactose, rhamnose, xylose, arabinose, as mono-, di-, and tri-glycosides (Brouillard, 1988; Mazza and Miniati, 1993). These sugars may be acylated with aromatic acids, such as *p*-coumaric, caffeic, ferulic, sinapic, gallic or *p*-hydroxybenzoic acids or aliphatic acids, such as malonic, acetic, malic, succinic or oxalic acid (figure 2.3)

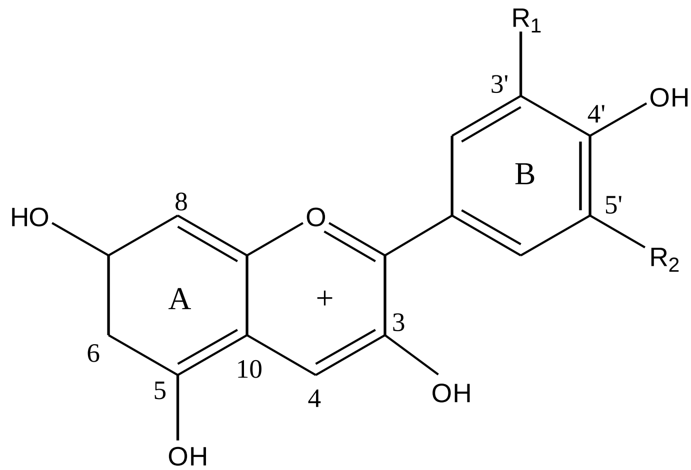
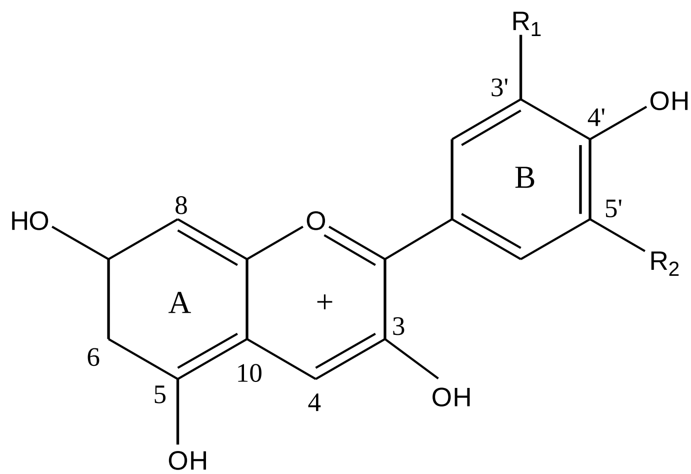


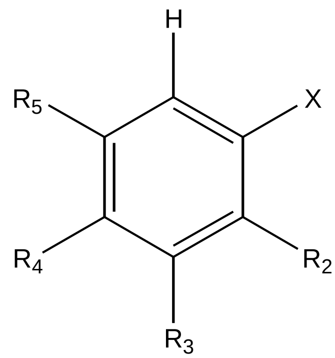
Figure 2.1: Basic structure of anthocyanin (Andersen and Jordheim, 2006)



	R_1	R_2
Pelagonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 2.2: Structures of the most common anthocyanidins occurring in nature (Andersen and Jordheim, 2006

Aromatic Acid



Common name	R_2	R_3	R_4	R_5
p-coumaric acid	H	H	-OH	H
ferulic acid	H	$-OCH_3$	-OH	H
sinapic acid	H	$-OCH_3$	-OH	-OCH
caffeic acid	H	-OH	-OH	H
p-hydroxybenzoic	H	H	-OH	H
gallic acid	-OH	-OH	-OH	-OH

Figure 2.3: Common organic acids acylated with sugar moieties (Sources: Robbins, 2003)

2.2.2. Physical and chemical properties of anthocyanin

Colour is generally evaluated by spectrophotometry. Isolated pigments have been studied by UV-visible spectroscopy. Anthocyanins have an intense absorption between 520 to 560 nm (visible region). Anthocyanins are polar molecules with aromatic rings containing polar substituent groups (hydroxyl, carboxyl, and methoxyl) and glycosyl (Delgado-Vargas et al, 2000). Therefore, various solvents such as alcohols, acetone, dimethyl sulfoxide, and water depend on the polar character of the anthocyanin molecule. Consequently, they are more soluble in water than in nonpolar solvents, but depending on the media conditions anthocyanins could be soluble in ether at pH value where the molecule remains unionized. These characteristics aid in the extraction and separation of anthocyanin compounds (Brouillard et al., 1982).

Due to the basic C6-C3-C6 anthocyanin structure, which is the source of colors formed by its chemical combination with glycosides and/or acyl groups and by its interaction with other molecules or media conditions (Brouillard et al., 1982), Considerable effort has been made to give explanations for the colour variations expressed by anthocyanins in plants, and in particular the blue colours (Brouillard and Dangles, 1994; Andersen and Jordheim, 2006). Four mechanisms, namely self-association, intramolecular co-pigmentation, intermolecular co-pigmentation between different molecules and complexation of anthocyanins with metal ions, have been suggested to stabilize the anthocyanins in the cell sap (Nerdal and Andersen, 1991). Co-pigmentation is supposed to be the most common mechanism in the formation of blue flower colours, and together with pH probably the most important factor influencing the flower colour (Brouillard and Dangles, 1993; Harborne and Williams, 2000). Moreover the shift to blue colours for polyacylated anthocyanins have also been explained by intra- or intermolecular co-pigmentation involving stacking between anthocyanidin and aromatic acyl moieties (Dangles et al., 1992, Honda et al., 2001). The bathochromic effects have been shown to depend on the number of aromatic acyl groups present and their linkage positions. Complexation with metal ions has shown to be efficient in influencing anthocyanin colour

Anthocyanins are far less stable than synthetic dyes and undergo structural transformations which end up with loss of colour. Considerations about anthocyanin stability are related to colour, equilibrium forms and co-pigmentation. The isolated anthocyanins are highly unstable and very susceptible to degradation (Giusti and Wrolstad, 2003). Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005). The chemical stabilisation of anthocyanins is the main focus of recent studies due to their abundant and potential applications, their beneficial effects and their use as alternative to artificial colorants (Rein, 2005).

2.2.3. Influence of structural on colour stability of anthocyanin

Chemical structure of anthocyanins plays an important role in their stability of anthocyanin. Chemical behavior of the pigment molecule can be affected by the substitution pattern of anthocyanidin, the number and placement of the hydroxyl and methoxyl groups in the aglycone. Also, glycosyl units and acyl groups attached to the aglycone, and the site of their bonding, have a noteworthy effect on stability and reactivity of the anthocyanin molecule, (Rein, 2005). Dao et al. (1998) reported that increased hydroxylation of the aglycone stabilizes anthocyanidin. Increasing methylation of the hydroxyl groups weakens the stability of the anthocyanins. (Mazza and Brouillard, 1987).

The substitution pattern of hydroxyl and methoxyl groups does not only affect the stability of anthocyanin but also the color appearance. As reported by Mazza and Brouillard (1987), when the number of hydroxyls increases, the color of anthocyanins changes from pink to blue. Methoxyl groups that replace the hydroxyls reverse the trend. Pelargonidin, cyanidin and delphinidin are less stable than peonidin, petunidin and malvidin due to the blocking reactive hydroxyl group by methylation (Andersen et al., 2004).

Acylation of anthocyanin can further increase colour stability (Bassa and Francis, 1987) Diacylation results in more intense color with a change in hue. P-coumaric acid induces more yellowish hue to pelargonidin 3-sophoroside-5-glucoside and ferulic acid a more bluish hue (Giusti et al., 1999).Anthocyanin with B-ring acylation produce stable and more intense coloration at pH values of 4 to 5.5 (Francis, 1989). Polyacylated anthocyanins are more stable than monoacylated anthocyanins and they possess high color stability throughout the entire pH range from acidic to alkali (Asen, 1972).

2.2.4. Influence of pH on colour stability of anthocyanin

Anthocyanin experiences dramatic colour changes in and undergo reversible structure transformation when its pH is change (Wrolstad et al., 2002). Brouillard, (1982) and von Elbe and Schwartz (1996) stated that anthocyanins exhibit greater stability under acidic condition at low pH values rather than in alkaline solutions with high pH values.

In acidic aqueous solution, four main equilibrium forms of anthocyanin exist, There are the the quinoidal anhydrobase, A (blue), the flavylium cation, AH+ (red), the pseudobase or carbinol, PB (colorless), and the chalcone, C (colorless or light yellow) (Chen and Hrazdina 1982). In a very acidic media (pH 0.5) the red flavylium cation is the only predominating equilibrium form. (Gonnet, 1998). Increasing pH therefore inflicts in decrease of both the color intensity and the concentration of the flavylium cation as it is hydrated by nucleophilic attack of water, to the colorless carbinol form. The carbinol form has lost its conjugated double bond between the A- and B-ring and therefore does not absorb visible light (Brouillard, 1982).

A more detailed report has been given by Giusti and Wrolstad, (2001). They reported that at pH value around 1, anthocyanins are mainly in the form of flavylium cations. There are predominated by the colourless hemiketal form when pH is increased to 4.5. The flavylium cation can be hydrolyzed rapidly at the 2-position by nucleophilc attack of water to give the colorless hemiketal form. As the pH increase above 4.5 carbinol form yields the colorless chalcone, through ring opening, At this point, the conjugated C-ring is destroyed and color is lost. As the pH continues to rise to 8 or above, the quinonoidal base ionized. Although in the alkaline state, the intensity of anthocyanins has been observed to increase (near pH 10) but the intensity is not as high as in acidic condition,In the alkaline state,anthocyanin also have diverse hue and λ_{max}, than in same solutions at pH 1. (Torskangerpoll and Andersen, 2005). Nevertheless, anthocyanins are identified to display a huge variety of color variations in the pH range from 1-14. The fact is, ionic nature of anthocyanin enables the changes in molecule structure according to the prevailing pH, resulting in variety of colors and hues at different pH values (Brouillard, 1982; von Elbe and Schwartz, 1996).

2.2.5. Influence of temperature and heat on colour stability of anthocyanin

Temperature is another factor that will affect anthocyanin stability. The degradation rate of anthocyanin increases with temperature especially during processing and storage (Palamis and Markakis, 1978). The degradation rates of anthocyanins also increased with increasing solid content during heating. This is because reacting molecules become closer when a product is concentrated (Patras, 2010).

Temperature increase at pH values from 2 to 4 induces the loss of the glycosyl moieties of anthocyanin by hydrolysis of the glycosidic bond.This leads to further loss of anthocyanin color, since the aglycones are much less stable than their glycosidic forms. The browning of the anthocyanin has been postulated the formation of a chalcone and first step in thermal degradation of anthocyanins (Markakis et al., 1982). Thermal degradation leads to brown products, especially in the presence of oxygen (Markakis et al., 1982). Study by Patras, 2010 reported that thermal degradation of anthocyanin is dependent on time and temperature of treatment and storage conditions, which increases with increasing storage temperature.This thermal degration follows first order kinetics (palamadis and Markakis 1978).

2.2.6. Influence of light on colour stability of anthocyanin

Apart from pH and temperature influence, intensity and stability of antocyanin pigment also depends on light exposure factor. Although light is necessary for the biosynthesis of anthocyanins, it also accelerates their degradation (Markakis, 1982). As Janna et al. (2007) revealed that daylight (or short wavelengths) and incandescent lamp (or long wavelengths) affect the degradation of the anthocyanins in different solutions. Thus, anthocyanins maintain their color much better when kept in the dark than in light. Abyari et al. (2006) reported that UV-irradiation speeds up anthocyanin degradation in four varieties of *Malus* regardless of pH. Similar results were also discovered by previous study by Palamidis and Markakis, (1978), who pointed out that the most vigorous anthocyanin loss (70%) was observed under fluorescent light at slightly elevated storage temperature. Furtado et al. (1993) revealed that the kinetic degradation of anthocyanin induced by light is similar to the kinetic degradation by heat, but the degradation of the flavylium cation fallows a different reaction of kinetic pathway. Abyari et al. (2006) found that the UV-irradiation degradation can be avoided by co-pigmentation with some phenolic acids. Setareh et al. (2007) also reported that the presence of co-pigments in the anthocyanin solution significantly prevented the degradation effect of UV-irradiation over a period of time.

2.2.7. Co-pigmentation of anthocyanin

Co-pigmentation of anthocyanin will enhance and stabilize the colour of anthocyanin. Co-pigmentation results in bluer, brighter and more stable anthocyanin pigment. It can be divided into intermolecular and intra-molecular co-pigmentation. (Yoshida and others 2000).

According to Brouillard, (1983), intermolecular co-pigmentation have been defined as the interactions between a colored anthocyanin and a colorless co-pigment which is not bound covalently to the anthocyanin molecule. Interaction involves instant π-π overlap, dipole-dipole interactions, and possible hydrogen bonding (Dangles and Brouillard, 1992). Cai et al (1990) pointed out that hydrogen bonding and hydrophobic interactions are the main reactions that lead to intermolecular co-pigmentation, resulting in 1:1 complex formation. Ionic of electrostatic interactions has also considered as potential means for intermolecular co-pigmentation (Chen and Hrazdina, 1981). Intermolecular interactions can occur with the flavylium cation and the quinonoidal base form of the anthocyanin (Asen et al., 1972; Williams and Hrazdina, 1979; Chen and Hrazdina, 1981). Interactions between flavylium cation and quinonoidal base with co-pigments, having the same structural features, inhibit nucleophilic attack of water on the anthocyanin molecule (Williams and Hrazdina, 1979)

2.3. Composition of coatings

Paint is a mixture of four basic ingredients: Pigments; Resins; Solvents and Additives (Weiss, 1997).

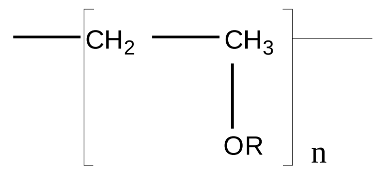
2.3.1. Resin

Resins are generally solid, sticky materials that hold the system together. They are also called binders, and when in solvent they are vehicle for the system (Tracton, 2006). Synthetic resins are viscous liquids capable of hardening. They are typically manufactured by esterification or soaping of organic compounds. The classic variety is epoxy resin, manufactured through polymerization-polyaddition or polycondensation reactions. Epoxy resin is twice stronger than concrete, seamless and waterproofing. Natural resins have been used since ancient times for a wide range of applications: varnishes, sealant, binding media and waterproofing. The varnish layer protects the paint film against dirt and mechanical damage, whilst at the same time achieving the proper colour saturation and gloss effects (Colombini, 2000)

Poly (vinyl alcohol) is a polymer that can act as a binder in coating technology. Poly (vinyl alcohol) can be prepared by hydrolyzing polyvinyl acetate in ethanol and potassium hydroxide. The acetate groups are hydrolyzed by ester interchange with methanol in the presence of anhydrous sodium methylate or aqueous potassium hydroxide. The polyvinyl acetate is in turn hydrolyzed to Poly (vinyl alcohol) via a base-catalyzed saponification reaction. The molecular weight of Poly (vinyl alcohol) is controlled through the polymerization step and generally is expressed in terms of a 4% solution viscosity. The viscosities are classified as ultra-low, low, medium, and high. The degree to which the polyvinyl acetate is converted to polyvinyl alcohol is referred to as the percent hydrolysis and is controlled during the saponification reactions. The percent hydrolysis is commonly denoted as super (99.3% conversion of vinyl acetate to vinyl alcohol), fully (98.0–98.8%), intermediate (95.5–97.5%), and partially (87.0–89.0%) hydrolysed (Boyland, 1964) .

The physical characteristics and its specific functional uses depend on the degree of polymerization and the degree of hydrolysis. PVA is classified into two classes namely: partially hydrolyzed and fully hydrolyzed. Partially hydrolyzed PVA is used in the foods. PVA is an odorless and tasteless. It is translucent, white or cream coloured granular powder. It is used as a moisture barrier film coating for food supplement tablets and for foods that contain inclusions or dry food (Saxena, 2004). Additionally the same author pointed out that PVA is also applied as a binding and coating agent. It is a film coating agent especially in applications where moisture barrier or protection properties are required. As a component of tablet coating formulations intended for products including food supplement tablets, PVA protects the active ingredients from moisture, oxygen and other environmental components, while simultaneously masking their taste and odour. It allows for easy handling of finished product and facilitates ingestion and swallowing. The viscosity of PVA allows for its application of the PVA coating agents to tablets, capsules and other application where solids content relatively high.

Boylant, (1997) reported that PVA provides excellent binding strength and improved ink-jet print quality versus typical latex binders. The viscosity developed depend upon the the pigment-to-binder ratio and PVA grade of chosen as a binder. PVA primarily controls the binding power for pigment adhesion and determines coating rheology. The structure of PVA shown in figure 2.4



where R=H or $COCH_3$

Figure 2.4: The structure of polyvinayl alcohol (partially hydrolyzed) *M.malabathricum* as source for natural colourant Co-pigmentation of anthocyanin

2.3.2. *M. malabathricum* as source for natural colourant

M. malabathricum of family Melastomataceae is investigated as a source of natural dye. *M. malabathricum* is a shrub that belongs to the family Melastomatacea and it is locally known as "pokok senduduk". It has beautiful purple colour flowers and slender undershrub with oblong leaves and has deep purplish blue fruits as seen in figure 2.5. Fruits of *M. malabathricum* are technically classified as berries. When the fruits are ripe, they break open and reveal the soft, dark purple, sweet but rather astringent-tasting pulp. Seeds are orange in colour (Wong, 2008).

The reddish stems and leaves of *M. malabathricum* are rough to the touch as they are covered with fine bristles. Each leaf is long and narrow and pointed at both ends. It has 3 distinct ribs and the fine bristles can be found only along on the ribs located on the leaf's underside. The attractive flowers produced by *M. malabathricum*, measuring up to 7 cm in diameter, are produced in a cluster at the tip of each shoot (Wong, 2008). The fruit is known to contain anthocyanins and tannins (Janna et al., 2006). Anthocyanins are natural, water-soluble, non-toxic colourant which suitable for wide range of applications. For the past decade, anthocyanins have become well known alternatives to synthetic dyes (Wong, 2008)

Figure 2.5: Fruit pulp of *M.malabathricum*

2.3.3. Additive

Additive is the substance added to the paint formula to improve a particular characteristic of the paint. The additive usually constitutes a small percentage of the paint and can improve properties of the paint, rheology and pigment wetting, or it can improve properties of the cured film such as corrosion resistance and UV durability (Florio and Miller, 2004).

Ferulic acid is an additive to prevent UV- irradiation since can absorb UV light. Ferulic acid is a universal plant constituent.it can be found in plant cell walls, leaves and seeds. It is made from the metabolism of phenylalanine and tyrosine. It occurs primarily in seeds and leaves. It can exist both in its free form and can be covalently linked to lignin and other biopolymers. Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential. UV absorption by ferulic acid catalyzes stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions. Ferulic acid is an effective deleterious radicals scavenges and can suppress radiation-induced oxidative reactions. Due to this, ferulic acid may serve as an important antioxidant to preserve physiological integrity of cells exposed to both air and impinging UV radiation. Similar photoprotection is afforded to skin by ferulic acid dissolved in cosmetic lotions. Its addition to foods inhibits lipid peroxidation and subsequent oxidative spoilage. By the same mechanism ferulic acid may protect against various inflammatory diseases. A number of other industrial applications are based on the antioxidant potential of ferulic acid (Ray et al.,2003)

CHAPTER 3: METHODOLOGY

3.1. Source of material

This chapter provides some details of the method of extracting natural colourant, purification of the colourant and also the formulation of paint systems using the extracts and polyvinyl alcohol (PVA). In this study, fruits pulp of *M. malabatricum* was chosen as the source of anthocyanin natural colorant. *M. malabatricum* used is wildly grown in Kelantan, Malaysia. To obtain a good quality extract, fully ripe fruits were collected and kept were kept at (-18 ± 2°C) before extraction was done. Trifluoroacetic acid (TFA) and methanol for anthocyanin extraction were procured from Sigma. PVA was used as a binder for coating preparation for this study was supplied by Sigma Aldrich.Distilled water used as solvent in order to prepared water-borne coating. Ferulic acid supplied by Sigma Aldrich was used as an additive in coating application.

3.2. Extraction of anthocyanin

50g of pulp of *M malabatricum* fruits was dissolved in 0.5% Trifluoroacetic acid (TFA) solution in methanol. The mixture was stirred at room temperature for 3 hours using a magnetic bar. The solutions were centrifuged for 15 minutes at 10,000 rpm. The supernatant liquids were then filtered using Whatman paper No 1 filter paper to remove any traces of residue. After extraction the extract was filtered, and the methanol was removed by evaporation under reduced pressure at relatively low temperatures (<30ºC). Figure 3.1 show the pictorial procedure of extraction of *M.malabathricum*

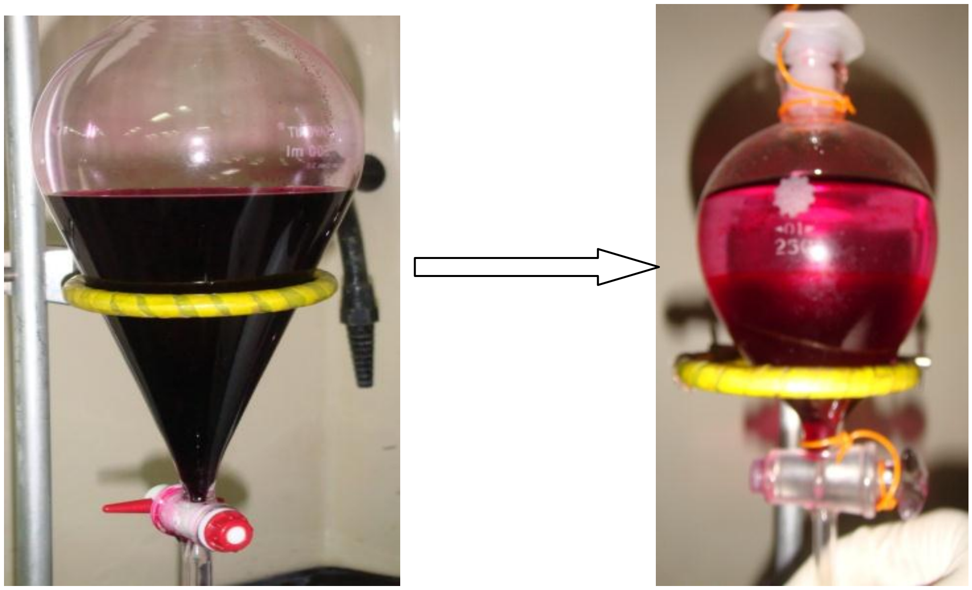
(a) Extraction of fruit pulp of *M.malabathricum*

(b) Fruit pulp of *melastoma* was dissolved in 0.5% trifluoroacetic acid (TFA) solution in methanol .Extraction was centrifudge for 15 minutes at 10, 000 rpm

Figure 3.1: Extraction of *M.malabatricum*

3.3. Purification of anthocyanin

After dissolving anthocyanin using 0.5 % TFA in methanol solution, the combined aqueous was evaporated in a vacuum evaporator for 2 days. The concentrates were then purified by liquid-liquid partition against ethyl acetate to remove chlorophylls, stilbenoids, less polar flavonoids and other non-polar compounds from the mixture (Andersen, 1988). After the separation, polar colourant were collected and again subjected to vacuum evaporator for 2 days. Pictorial procedures are shown in figure 3.2



Separation Polar and non-polar in separating funnel

After the separation, polar pigment were Collected**.**

Figure 3.2: Purification of anthocyanin by Liquid-liquid partition

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After the liquid-liquid partition, the polar colourant concentrate which include anthocyanin and other impurities were removed by using Amberlite XAD column chromatography. The column containing extract colourant was then washed several times with pH 7 distilled water in order to remove sugar and aliphatic acids. The column was washed again with 50% acidified methanol containing (0.5 % v/v) TFA. After this step, the column was washed with absolute methanol to further removed absorb anthocyanin from amberllite column and the filtrate collected. This step repeated until the filtrate become clear which means all pure anthocyanin has been collected. Then the column again washed with 50% acidified methanol containing (0.5 % v/v) TFA and distilled water at pH 7. The collected purified samples was subjected to evaporation process for two days under reduced pressure at relatively low temperatures (<30ºC)

3.4. Sample preparation

3.4.1. Crude and purified colourant from fruit pulp of *M.malabathricum*

Ferulic acid (co-pigment) was added at five different percentages (1%, 2%, 3%, 4% and 5% FA) in order to improve the colour stability of anthocyanin crude and purified colourant. Another set sample were prepared to study the effect of pH on crude and purified anthocyanin by adjusting the pH, (initial pH, pH 1.pH 3.pH 5, pH 7, pH 9,pH 11). The pHs of crude and purified anthocyanin colourant were adjusted by adding different amount of 1M HCL and 1M NaOH. The variation of colours obtained and the stability of colour crude and purified anthocyanin colour at different FA content and different pHs were determined using Commission Internationale de l'Eclairage, (CIE system),CIE colour analysis. The percentage of FA added that exhibit the best stability were then determined. On obtaining the right FA percentage at different pH, (pH 1.pH 3.pH 5, pH 7, pH 9, pH 11). Colour analysis and stability was again performed using CIE colour analysis.Samples were prepared in triplicate.

3.4.2. Crude and purified Anthocyanin-PVA blend from fruit pulp of malabathricum

The crude colourant was mixed with 30% poly (vinyl) alcohol (PVA) to form a coating system. Then again the steps followed during the preparation sample in crude anthocyanin above were repeated for Anthocyanin-PVA blend for both crude and purified. All samples were also prepared in triplicate.

3.5. Colour Analysis by using CIE sysyem

3.5.1. Colour analysis measurements

For colour analysis study, the samples were added to transparent glass bottles with screw cap.Then samples in the glass bottle subjected to 100% (17.55 lux) UV-B irradiation for 93 days of exposure. While, for crude and purified anthocyanin-PVA blend, the liquid samples were coated on glass slide kept overnight in dark for curing before exposed to Lux intensity of 100% (17.55 lux) UV-B irradiation for 93 days exposure. These samples were exposed to UV-B irradiation by placing them under UV lamp which emitted radiation at 312 nm. The distance between the samples and the light source was fixed at 5 cm. The Spectral curves were recorded with a Shimadzu 3101 spectrophotometer (regular transmission, from 380 to 780 nm with a 2 nm bandwith) in 10 mm optical path quartz cuvettes by using colour analysis software, Commission Internationale de l'Eclairage, (CIE system).

3.5.2. Colorimetric calculation

This study focused on colorimetric calculation by using CIE system in order to analys the colour stability of samples.According to Birse (2007) the use of absorbance profiles and $\boldsymbol{\lambda}_{max}$ values are non-intuitive and can be difficult for an inexperienced person to understand. This is because the $\boldsymbol{\lambda}_{max}$ value requires an understanding of absorbance values, wavelengths and colours before making an adequate judgement. Furthermore, absorbance profile that were being presented may not be straightforward; as varying degrees of absorbance at different wavelength may imply that the colour observed is not simply red or orange. For example, in addition to high red absorbance, different proportions of absorbance in the yellow, green and violet regions of the visible spectrum may indicate that a red-brown colour is observed. CIELab colour values are a more appropriate measurement for the colour of natural colourant, as the system can be used to describe all the colours visible to the human eye. Thus, colours can be precisely described using CIELab colour co-ordinates. The method for doing this was introduced in 1931 by the international standards agency Commission Internationale de l'Eclairage, CIE. To measure the variables that create color sensations, the CIE established a reproducible, spectrophotometry based, device-independent color model constructed from a light source, an observer, and an object. The results of a CIE-compliant measurement and transformation are coordinates that locate the specimen in a horse-shoe-shaped color space representing human colour perception.

From the transmittance spectral curves, the X, Y and Z tristimulus values were computerized for a couple of CIE illuminant/observer conditions: D65 (diffuse daylight type) and A (tungsten light), both for the 'suplementary" or 2°, CIE observer, according to the weighted ordinate method. L*, a* and b* are calculated from the tristimulus value (X, Y, Z) which are the backbone of all colour mathematical models. The location of colour, in the CIELAB colour space, is defined by a three dimensional cartesian (rectangular) co-ordinate system.Along the vertical axis, L* is a measure of lightness from completely opaque (0) to completely white (100). Simply, the L* value can be used to describe the lightness of the colour. The hue circle, used to describe the colour in the horizontal plane where a* is a measure of redness (or –a* of greenness and b* is a measure of yellowness (or –b* of blueness) (Figure 3.3). On the chromatic circle in figure 3.3 below hue angle values are stepped counterclockwise from h_{ab} O$^{\circ}$-360° (magenta-red) across a continuously fading hue circle, the other remarkable values of which are 90° (yellow), 180° (bluish-green) and 270° (blue).

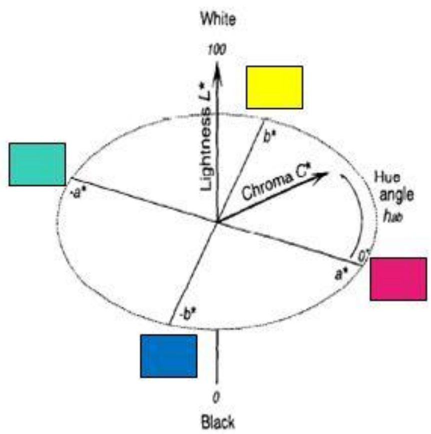


Figure 3.3: CIELab colour space describing colour in three dimensions, luminance, L*, the red- green axis, a*, and the blue-yellow axis, b*

The saturation or chroma corresponds to the brightness of the colour and is generally observed by how intense the colour is. The chroma is derived from a* and b* co-ordinates, and is calculated using Pythagoras' theorem (equation 3.1). While, hue angle (equation 3.2), is calculated from a* and b* values using trigonometric ratios as in Figure 3.4

$$C^* = \sqrt{a*^2 + b*^2} \qquad \textbf{equation (3.1)}$$

$$H^\circ = \tan^{-1}(a * | b *) \qquad \textbf{equation (3.2)}$$

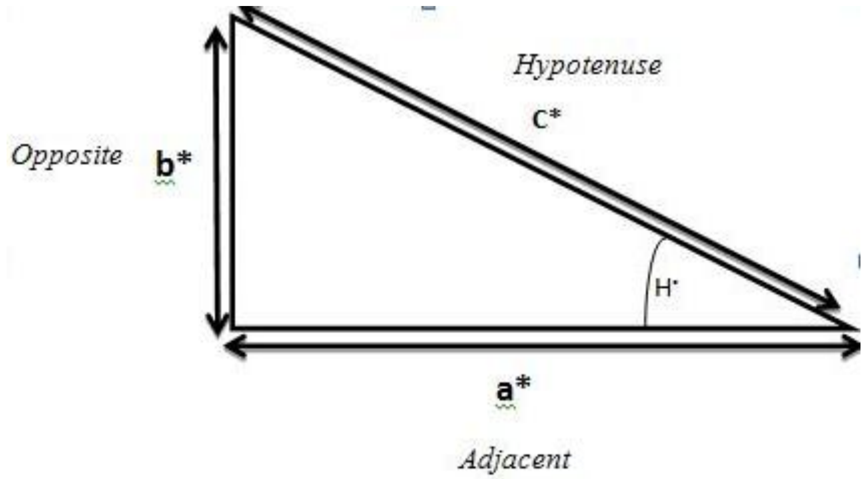


Figure 3.4: Trigonometric relationship involving the known sides a* and b* used to derive the Chroma, C* and hue angle, H◦ respectively

Two additional values presented in this thesis are derived from CIELab colour coordinates are colour difference and saturaturation denoted by ΔE and s by using equation below:

$$\Delta E= (\Delta L +\Delta a+\Delta b)^{1/2} \qquad \text{equation (3)}$$

$$S=C^*/ L^* \qquad \text{equation (4)}$$

3.6. Experimental design and statistical analysis

A completely randomized design with three replications was used. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences). Multifactor analysis of variance was applied with source of variance and color measurement instruments. Differences between means were tested using analysis of variance (ANOVA) based on Duncan test with level significant of $P<0.05$.

CHAPTER 4: RESULTS OF CIE COLOUR ANALYSIS FOR ANTHOCYANIN COLOURANT

4.1. Introduction

Chapter 4 gives detailed investigation of stability and colour analysis study of the crude and purified anthocyanin colourant from *M. malabathricum* for a coating system. This chapter begins with the colour variation of crude and purifiied colourant of *M. Malabathricum* during storage under UVB – irradiation (100% lux intensity) for 93 days of storage in order to study effect addition of Ferulic Acid (FA) as stabiliser on colour visual variation by using CIELAB colour analysis. And effect of pH to the crude and purified colourant also studied. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences).Differences between means were tested using analysis of variance (ANOVA) with significant of $P<0.05$ level. The statistical methods used for the data analysis were two-way analysis of variance (ANOVA) to find out whether there is a relationship between percentage of FA and pH variation on visual colour variation

4.2. Colour Analysis of Crude anthocyanin colourant from Fruit Pulp of *M. malabathricum*

4.2.1. Influence of different percentage of FA added on Visual Colour Variation

Figure 4.1 present influence of different percentage of FA addition for crude fruit pulp of anthocyanin *M. malabathricum* colourant on the values of the colour parameters (colorimetric indexes and CIELAB variables) in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (blueness and yellowness). It can be shown that at zero time strorage, the non-enhance crude anthocyanin colourant (0% FA) resulted in the lightest samples with highest L* (64.654 ± 0.017). However addition of FA (1%, 2%, 3%, 4% and 5% FA) significantly decreased the L* value and samples with the addition of 3% FA showed the lowest L* value (49.998 ± 0.010) followed by 2% FA added (55.761 ± 0.170). The lightness of the non-enhance crude anthocyanin colourant slightly increased upon storage under 100% lux intensity (17.55 lux), with the end of storage (3 month) the L* value recorded was 80.597± 0.016). These results revealed that the colour of non-enhance samples lighter after 3 month of storage compared to the samples containing FA. Furthermore the colour stability of crude anthocyanin improved with the addition of 3% FA with the lightness, L* of the sample decreased in 1 and 2 month of storage, whereas an insignificant increased in L* value was observed during the last period of samples storage (55.896 ± 0.009).

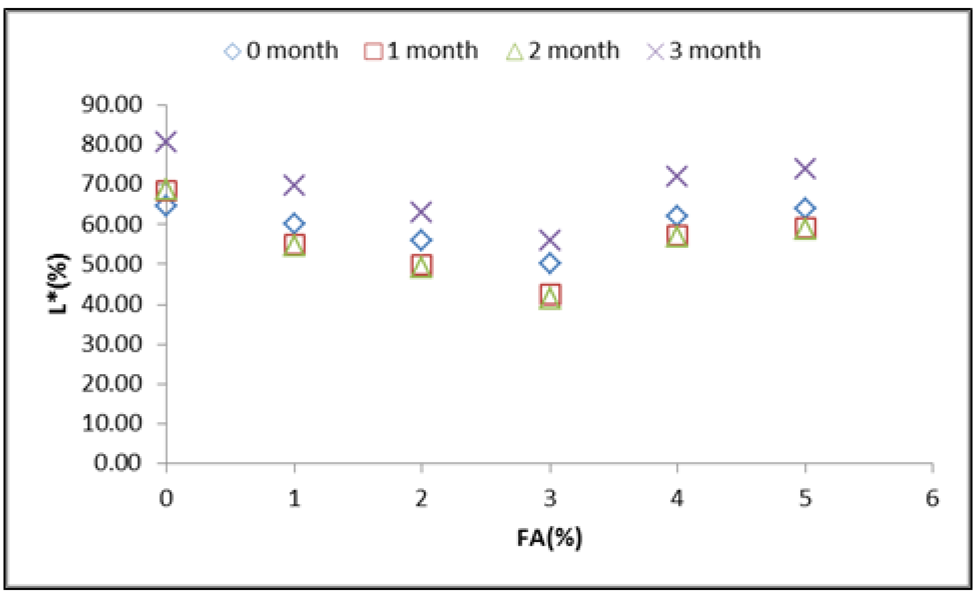


Figure 4.1: Relationship between percentage of FA (%) and L* values (%) for crude *M. malabathricum* anthocyanin colourant during 3 month of storage

Furthermore, different addition of FA percentage also affects the colour chromaticity, C* values during 3 month of storage. As shown in figure 4.2, in the beginning of storage, the C* of non-enhance samples resulted in dull colour with the lowest C* value (18.285 ± 0.0153). On the other hand, addition of FA successfully increased the C* value with resulted in brighter colour. According to the table below, crude anthocayanin colourant with the addition of 3% FA gave the brighter colour with highest C* value (31.941 ± 0.006) compared to the other samples tested. Besides that as seen in table further increased in FA % addition up to 4 and 5% FA resulted in decreased of C* value (21.393 ± 0.019) and (20.970 ± 0.010) respectively .The chroma results for crude anthocyanin colourant was observed to decreased over the storage period for non-enhance samples.Furthermore the C* for crude antghocyanin colourant exhibit slightly increased upon storage up to 2 month before expericed decreased in C* at the end of the storage.This trend was obviously for 3% FA added crude colourant which the C* value increased over 2 month of storage (40.804 ± 0.006), however prolong the storage up to 3 month resulted in significantly decreased in C* value (27.620 ± 0.004). The results gained for this investigation showed that 3% FA significantly enhance the colour of crude anthocyanin colourant by increased the C* value at the beginning of storage. Nevertheless, as non-enhance sample at the end of storage, the colour of 3% FA added samples also faded with resulted in decreased the C* value. On the other hand the end of stprage, 3% FA added still resulted in the highest C* (27.620 ± 0.004) value which means more coloured compared to the others.

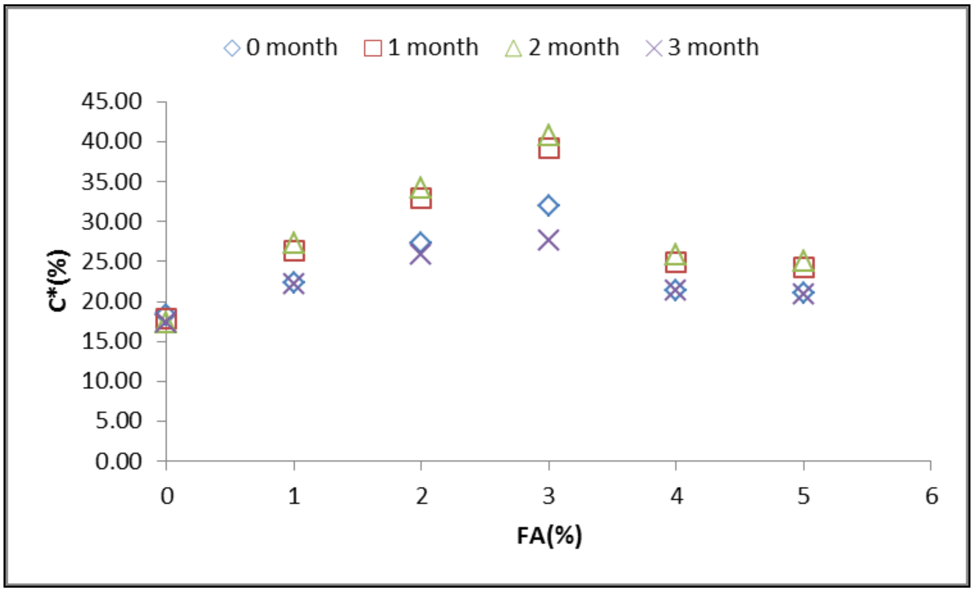
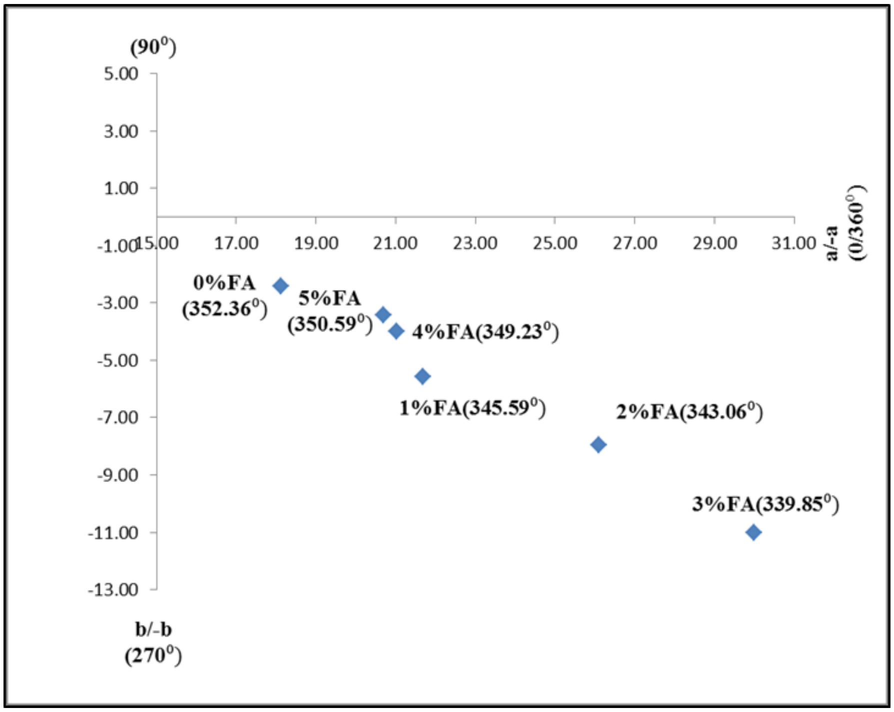


Figure 4.2: Relationship between percentage of FA (%) and C* values (%) for crude *M. malabathricum* anthocyanin colourant during 3 month of storage

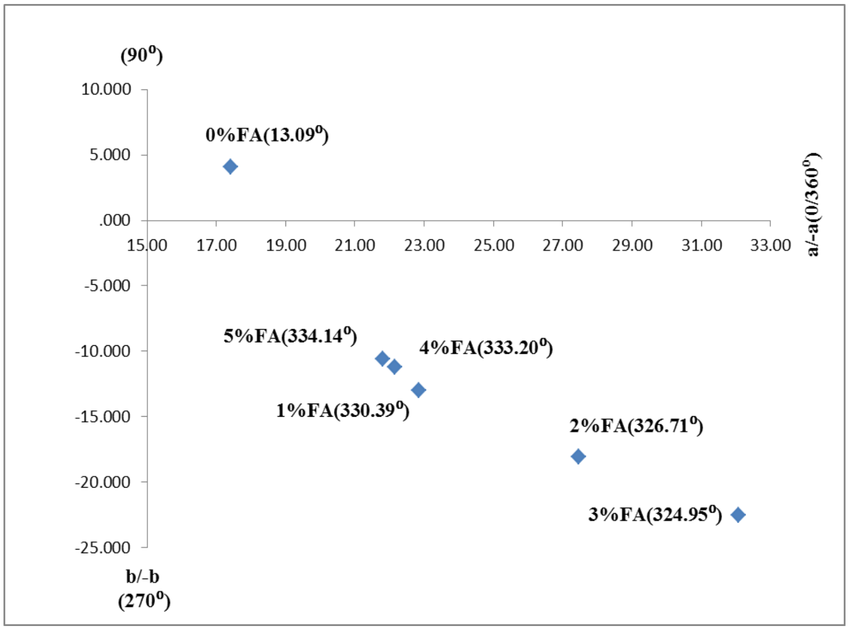
Moreover, hue also the other parameter that influenced by different of percentage FA added during 3 month of storage. As seen in figure 4.3(a), the initial colour position on the circle recorded for non-enhance crude anthocyanin colourant with the H^o (352.360 ± 0.012), then the hue angle first moved to the lower value (counter clockwise) with the addition of 1% FA (345.590 ± 0.018) to 3% FA (339.850 ± 0.008). On the hand, it can visibly note that the non-enhanced sample for crude anthocyanin colourant from fruit pulp *M.malabathricum* present in a* value (-18.123 ± 0.015) and negative b* (-2.431± 0.014) with hue angle (352.360 ± 0.012) at zero time of storage. However, addition of FA significantly increased the blue colour, with resulted in more negative b* value since b* measures blueness when negative. According to the figure 4.3(a) also, it can be realized that addition of 3% FA gave better enhancement with resulted in positive a* value and more negative b* value (-11 ± 0.012) with the H^o (349.230 ± 0.012).

Furthermore, the colour of non-enhance sample (0 % FA) experience decreased in H^0 to the lower value during 3 month of storage can be noted. It can be realized that, at the first month figure 4.3(b) and second month of the storage, figure 4.3(c), H^0 significantly decreased to (13.093 ± 0.021) and (15.551 ± 0.017) .While at the end of storage, Figure 4.3(d), the a* value decreased to (1.971 ± 0.016) and the b* increased to more yellowness value (17.198 ± 0.016) with the H^0 (83.462 ± 0.012).Nonetheless, addition of 3% FA resulted in increased the colour stability of crude colourant by a* value recorded at the end of storage was 25.463 ± 0.004 and b* 10.702 ± 0.006 with the H^0 (22.796 ± 0.010).



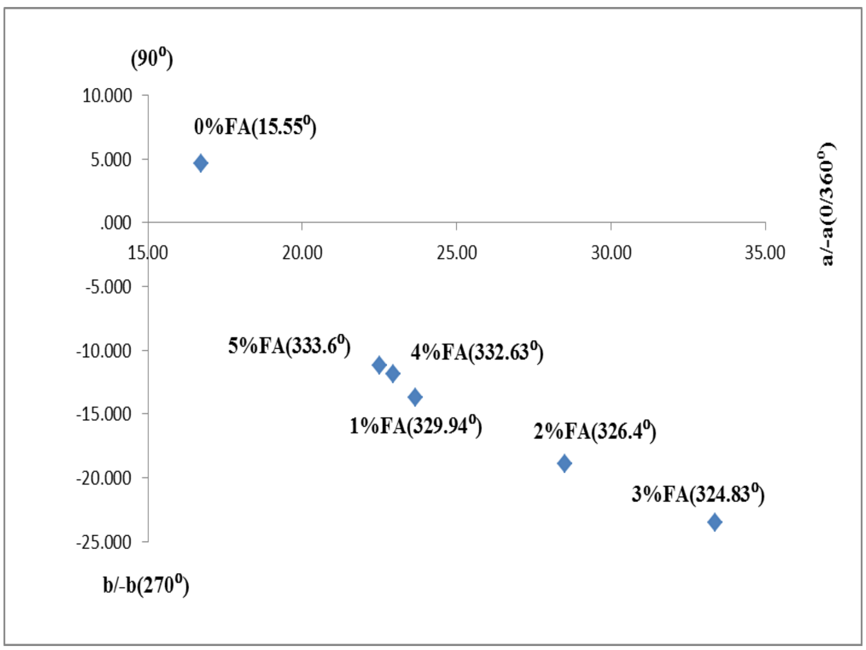
(a)

Figure 4.3: Relationship between percentage of FA and H◦ with a*b* co-ordinate for crude *M. malabathricum* anthocyanin colourant during (a) 0 month of storage, (b) 1 month (c) 2 month and (c) 3 month of storage



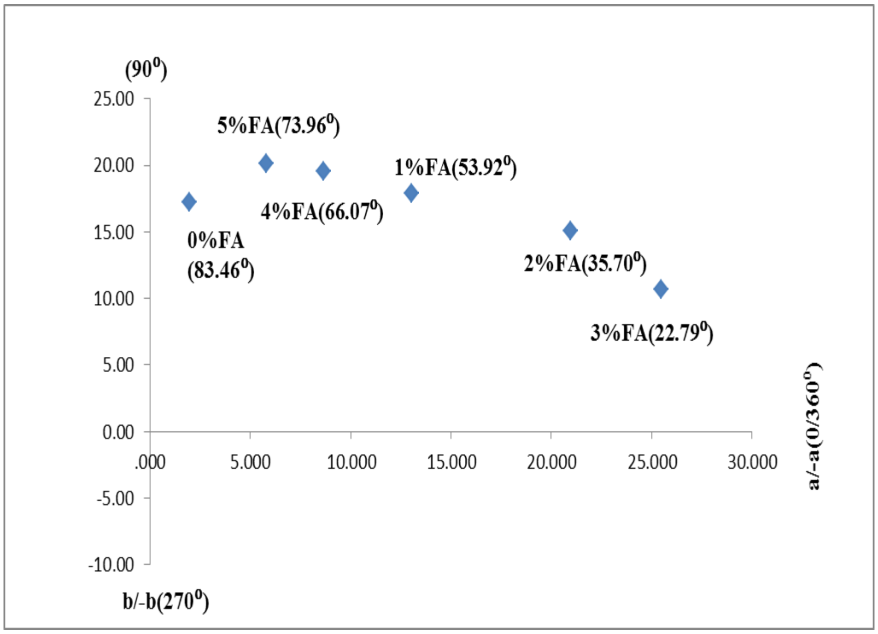
(b)

'Figure 4.3 continued'



(c)

'Figure 4.3, continued'



(d)

'Figure 4, continued'

Table 4.1: Influence of FA addition on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin colourant from *M.malabathricum*

FA (%)	**TIME (Month)**					
	0	**1**	**2**	**3**	**ΔE_1**	**ΔE_3**
0	s_0=0.2828	s_1=0.2619	s_2=0.2532	s_3=0.2148	ΔE_1=7.469	ΔE_3=30.006
1	s_0=0.3731	s_1=0.4779	s_2=0.5007	s_3=0.3183	ΔE_1=9.019	ΔE_3=26.810
2	s_0=0.4894	s_1=0.6601	s_2=0.6973	s_3=0.4108	ΔE_1=11.806	ΔE_3=24.635
3	s_0=0.6388	s_1=0.9262	s_2=0.9808	s_3=0.4941	ΔE_1=13.884	ΔE_3=22.940
4	s_0=0.3451	s_1=0.4340	s_2=0.4545	s_3=0.2972	ΔE_1=8.718	ΔE_3=28.314
5	s_0=0.3288	s_1=0.4101	s_2=0.4276	s_3=0.2840	ΔE_1=8.618	ΔE_3=29.597

Table 4.1 presents the Total Colour difference (ΔE) of crude anthocyanin colourant with and without addition of FA, which showed the combination of the changes of three components (Chroma, hue and light). As seen in table, this changes in choromatic parameters during storage were summarized by calculating the (ΔE). Highest (ΔE) was observed for the samples with the addition of 3% FA followed by 2% FA at beginning of storage with the (ΔE) recorded was ΔE_1=13.884 and ΔE_1=11.806 respectively. And again can be observed that non-enhance sample (0 %FA) resulted in the lowest ΔE_1=7.469 in the beginning of storage. In contrast at the end of storage the non-enhanhce gave the highest total colour difference, ΔE_3=30.006. Small increment in ΔE_3 was observed for the sample with the addition of 3% FA. ΔE_3=22.940 followed by 2% FA, ΔE_3=24.635.

In addition, the saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. The results gained for this analysis of saturation displayed in table 4.1. As observed in table above, the highest colour saturation recorded for the samples with the addition of 3 % FA at the beginning of storage, s_1=0.9262 and further increased up to s_2=0.9808 in the second month of storage. It showed that crude anthocyanin colourant with 3% FA was the most coloured sample in this investigation. However, as realized in the table non-enhance sample (0% FA) showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.2828 to s_3=0.2148 at the end of the storage. Hence it directly noticed that, the colour of untreated sample obviously faded during the 3 month of the storage.On the other hand, at the end of the storage, the 3% FA added samples gave the most coloured samples with highest saturation s_3=0.4941 compared the other samples tested.

4.2.2. Influence of different pH on Visual Colour Variation of crude anthocyanin colouranInfluence of different

Figure 4.4 displays the influence of different pH (pH initial 5.6, pH 1,3, 7,9 and 11) on visual colour variation for crude fruit pulp of anthocyanin *M. malabathricum* colourant in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness).It can be seen that starting from pH 1 the lightness percentage of *M. malabatricum* (64.098±.014) decrease over increasing pH 11 (57.643±.018) at zero time of storage. However, when approaching pH 5.6, the lightness started to increase (64.654±.016) before decrease when pH reaching pH 7 (62.511±.014) and continue following the decreasing trend.

During the storage period, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (89.532±0.019) after the 3 month of the storage. As expected, the slightly increased in L* value was observed in the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (77.907±.014), (77.004±0.016) and (76.144±.013) respectively.

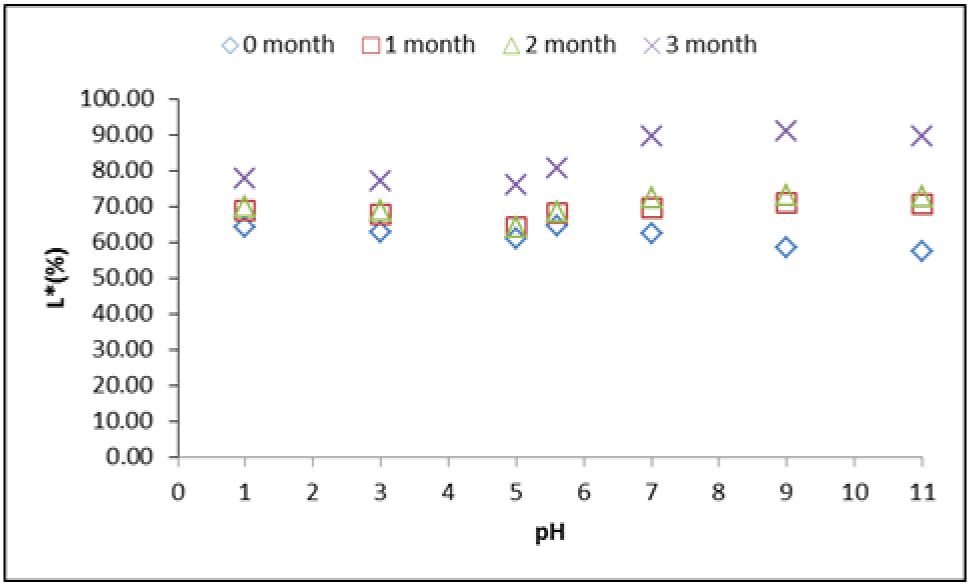


Figure 4.4: Relationship between pH variation and L* values (%) for crude *M. malabathricum* anthocyanin colourant during 3 month of storage

Furthermore, altered the pH for crude anthocyanin colourant also affected the colour chromaticity. As seen in figure 4.5, the C* value for most acidic crude anthocyanin colourant pH1 (48.561±0.012) recorded as the highest chroma compared to the other sample. However, It can be seen that, as starting pH 1 the chromaticity decrease (48.561±0.012) with increasing pH 11 (15.355±0.018) nevertheless when reaching pH 9 the chromaticity were slightly increase (34.750±0.010).On the hand, the chroma of the crude anthocyanin colourant also decreased during 3 month of storage which result in dull in colour. Most coloured sample pH 1 also experience the colour fading by reducing in C* value over time and it obviously at the end of the storage, the C* value recorded was (42.922±0.014) which still resulted in the highest C* value (brighter colour) compared to the other pH study. Same tendency was also obsereved for the origin sample without adjusted pH which the C* value are also decreased over the storage period, and showed better result compared to alkaline crude colourant pH 11(11.152 ±0.014). Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

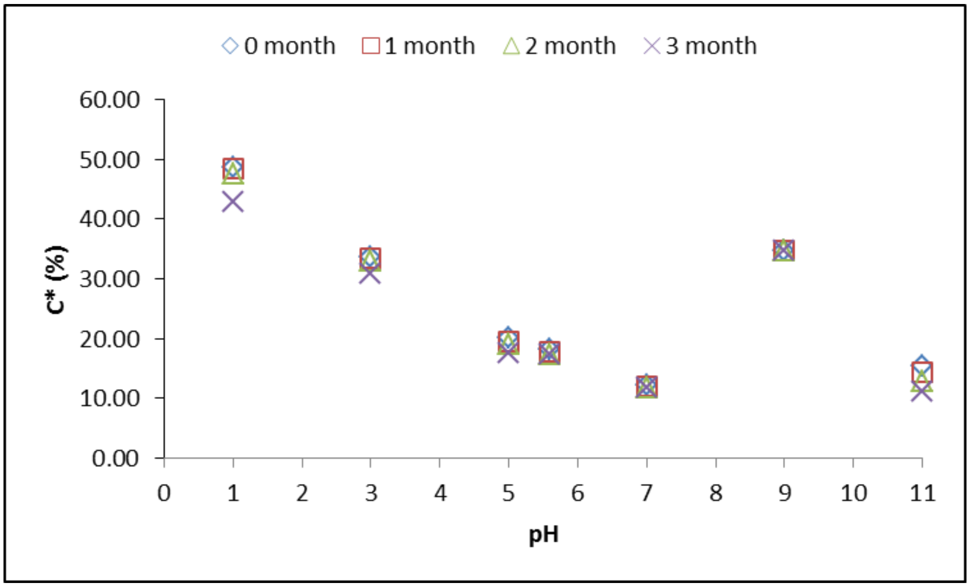
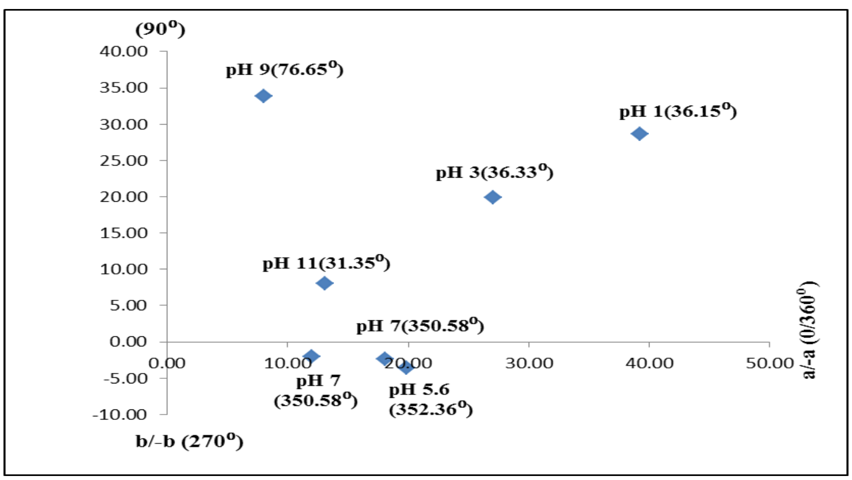


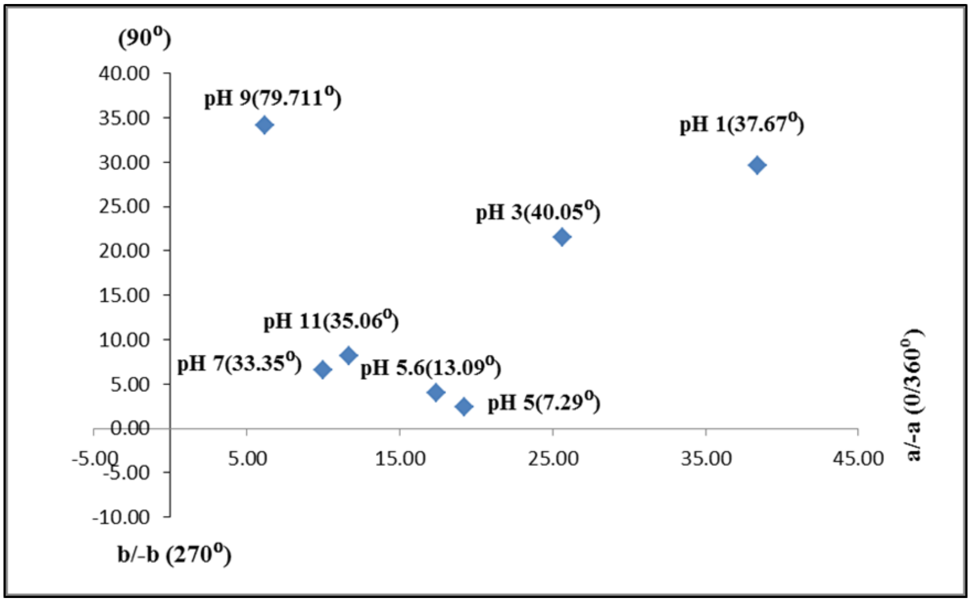
Figure 4.5: Relationship between pH variation and C* values (%) for crude *M. malabathricum* anthocyanin colourant during 3 month of storage

Hue, which also vital affect caused by variation of pH. As figure 4.6 (a) to (d), further augmentation of pH 1, H^{o} (36.148±0.013) to higher pH 5.6 caused an important counterclockwise shift of hue angle H^{o} (352.360±0.012), meaning that the hues now moved back to yellower tonalities. Based on figure 4.6(a), it shows that the pH 5.6 of *M.malabathricum* present a* values (18.123 ± 0.015) and negative b* values (-2.431 ± 0.014) with hue angle (h_{ab} 352.360 ± 0.012) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (39.213 ± 0.012) and negative b* values moved to positive value (28.646± 0.018) while hue angle moved clockwise to lower value (h_{ab} 36.148^{o} ± 0.013) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (8.021±0.015) and b* slightly increase to positive values (33.812±0.013) while hue angle moved counterclockwise H° (76.654±0.016). Furthermore, the pH variation also affects the visual colour stability of crude anthocyanin colourant during storage. Based on the figure 4.6(a) to figure 4.6(d), it clearly noticed that the visual colour of samples with more alkaline pH easily faded during storage with the a* (1.005±0.017) moved to lower positive value and b* slightly moved to more yellower tonalities (7.989±0.018), while the hue angle moved counterclockwise H° (84.829±0.016) which showed the colour degradation of anthocyanin. Additionally, the most coloured sample at pH 1 also experience calour degradation durig 3 month of the storage with the a* values started to move to the less positive (20.400±0.014) and b* decreased to more positive value (37.765±0.016) with increasing the H° (61.622±0.015). Nonetheless it again can be noticed that, at the end of the storage, Figure 4.6(d) the pH 1 successfully improved the colour stability of the crude colourant which resulted in the most coloured sample compared to the other pH study.



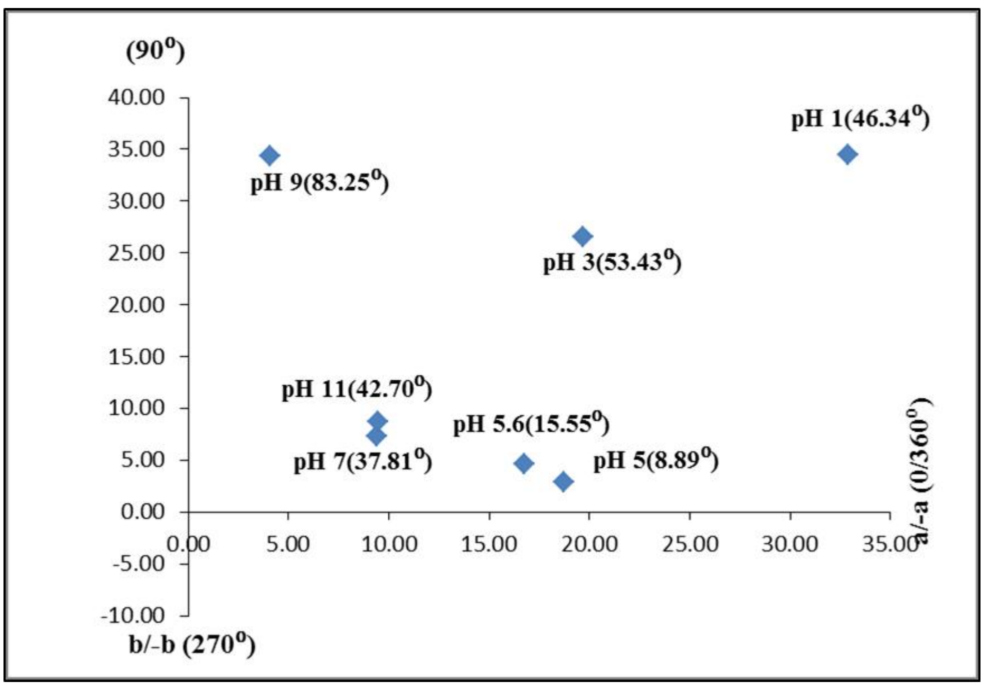
(a)

Figure 4.6: Relationship between pH variation and H° with a*b* co-ordinate for crude *M. malabathricum* anthocyanin colourant during 0 month of storage



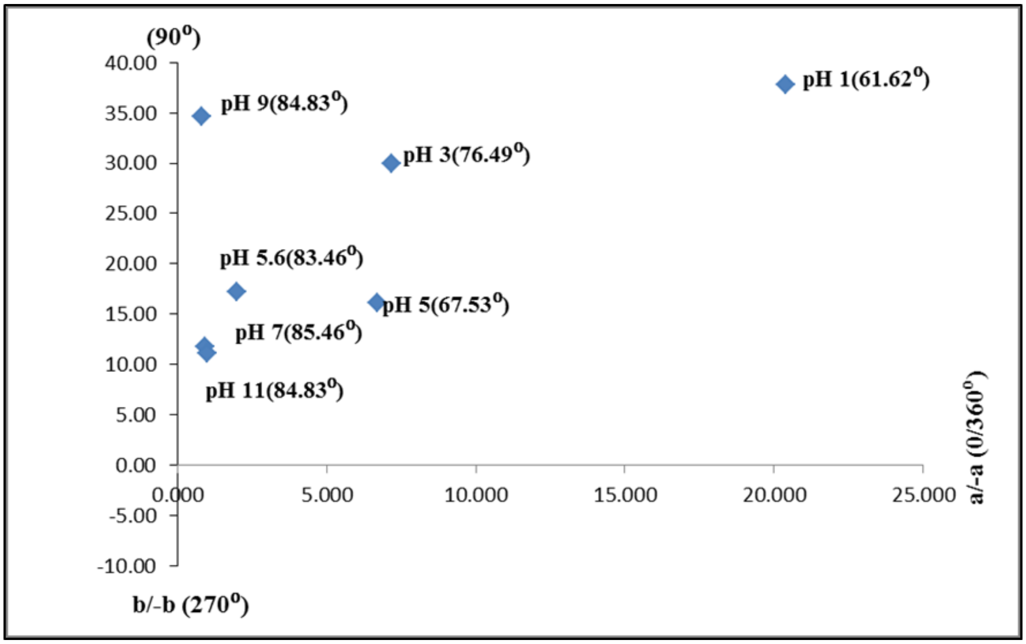
(b)

'Figure 4.6, continued'



(c)

'Figure 4.6, continued'



(d)

'Figure 4.6, continued'

Table 4.2: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin colourant from *M.malabathricum*

pH	TIME (Month)					
	0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.7576	s_1=0.7036	s_2=0.6814	s_3=0.5509	ΔE_1=4.991	ΔE_3=25.055
pH 3	s_0=0.5350	s_1=0.4932	s_2=0.4798	s_3=0.3996	ΔE_1=5.567	ΔE_3=26.410
pH 5	s_0=0.3308	s_1=0.3025	s_2=0.2950	s_3=0.2299	ΔE_1=6.771	ΔE_3=28.115
pH 5.6	s_0=0.2828	s_1=0.2619	s_2=0.2532	s_3=0.2148	ΔE_1=7.469	ΔE_3=30.006
pH 7	s_0=0.1946	s_1=0.1723	s_2=0.1643	s_3=0.1331	ΔE_1=11.750	ΔE_3=32.331
pH 9	s_0=0.5925	s_1=0.4878	s_2=0.4733	s_3=0.3802	ΔE_1=12.620	ΔE_3=33.135
pH 11	s_0=0.2664	s_1=0.2025	s_2=0.1772	s_3=0.1246	ΔE_1=12.993	ΔE_3=34.253

Table 4.2 displays the Total Colour difference (ΔE) of crude anthocyanin colourant with different pH (pH initial 5.6, pH 1, 3, 7, 9 and 11). Since the a* and b* parameters represent the redness and the yellowness on the chromaticity dimension, while the c* and h° parameter represent the Hunter a* and b* parameters and the ΔE represents the colour change of three colour coordinate (C*, L* and h) of sample before and after exposed to high UV-B irradiation for 3 month. Smallest (ΔE) was noticed for the samples with pH 1 ΔE_1=4.991 at the beginning of the storage. After 3 month of the storage the ΔE_3=25.055 was increased for pH 1 which showed the colour varied compared to the first month. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample at pH 11 (ΔE_1=12.993)**.**Similarly at the end of the storage pH 11 gave the highest total color change compared to the other samples tested (ΔE_3=34.253).

In addition, the saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. The results gained for this analysis of saturation displayed in table 4.2. As observed in table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.6, pH 1, 3, 7, 9 and 11). Highest colour saturation recorded for the samples at pH 1 (s_0=0.7576) and gradually decreased during storage with the saturation recorded were s_1=0.7036, s_0=0.6814, s_2=0.5509 correspondingly. Hence these results showed that pH 1 resulted in more saturated red colour compared to the other acidic pH. Nevertheless the decreasing of the saturation over 3 month of storage period showed that pH 1 also degraded. However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.2664to s_3=0.1246at the end of the storage. Therefore it directly perceived that, the colour of sample at pH 11 obviously faded during the 3 month of the storage.

4.2.3. Influence of different pH on Visual Colour Variation of crude anthocyanin colourant containing 3% FA

Influence of different pH (pH initial 5.4, pH 1, 3, 7, 9 and 11) on visual colour varion for crude fruit pulp of anthocyanin *M. malabathricum* colourant containing 3% FA displays in figure 4.7. Parameter in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) was observed.It can be seen that the lightness percentage decreased with increased in pH from pH 1 (53.706 ±0.008) to pH 11 (42.655±0.009) at zero time of storage. Conversely, when approach pH 5.4, the lightness started to slightly increase (49.997±0.0095) before underwent to decreased when pH approach pH 7 (47.726±0.0121). According to this plot, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (67.768±0.009) after the 3 month of the storage. As expected, the slightly increased in L* value was observed for the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (64.599±0.0107), (52.109±0.0058) and (48.973±0.008) respectively. As accordance to the results obtained, the colour stability of the crude anthocyanin colourant degraded during the storage period under UVB- irradiation by increasing the the L* value which was resulted in lighter colour and the end of the storage pH 3 presents the most colour sample with the lowest L* value (48.973±0.008)

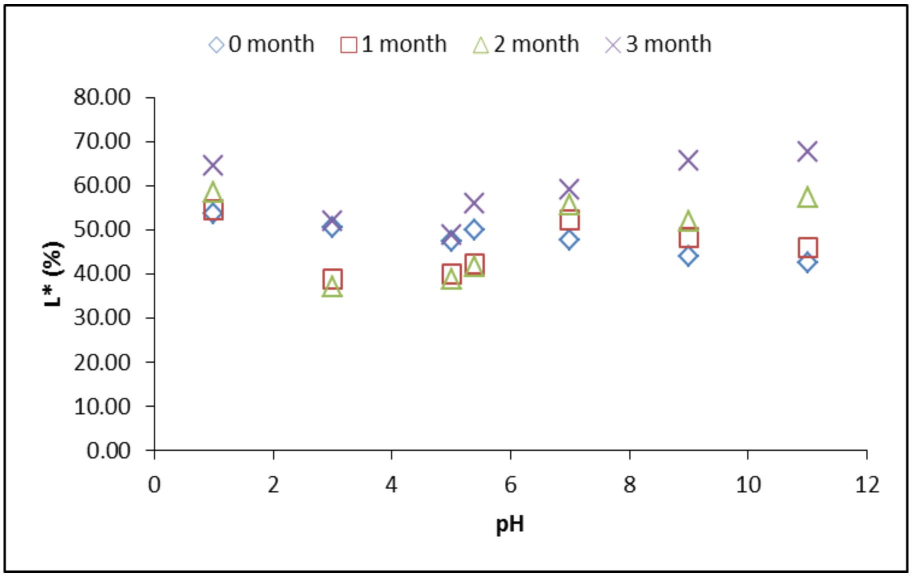


Figure 4.7: Relationship between pH variation and L* values (%) for *M. malabathricum* with 3%FA during 3 month of storage

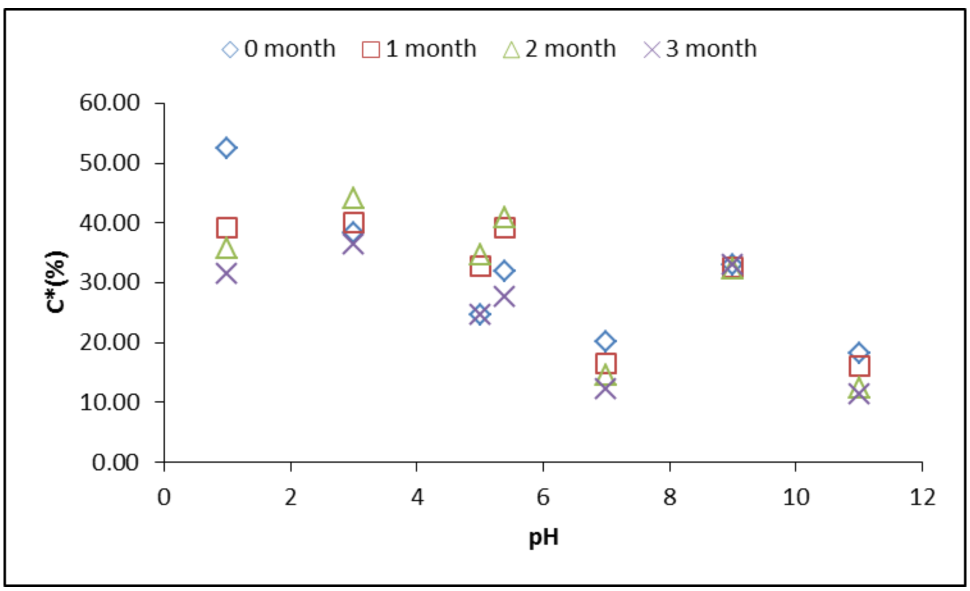


Figure 4.8: Relationship between pH variation and C* values (%) for *M. malabathricum* with 3%FA during 3 month of storage

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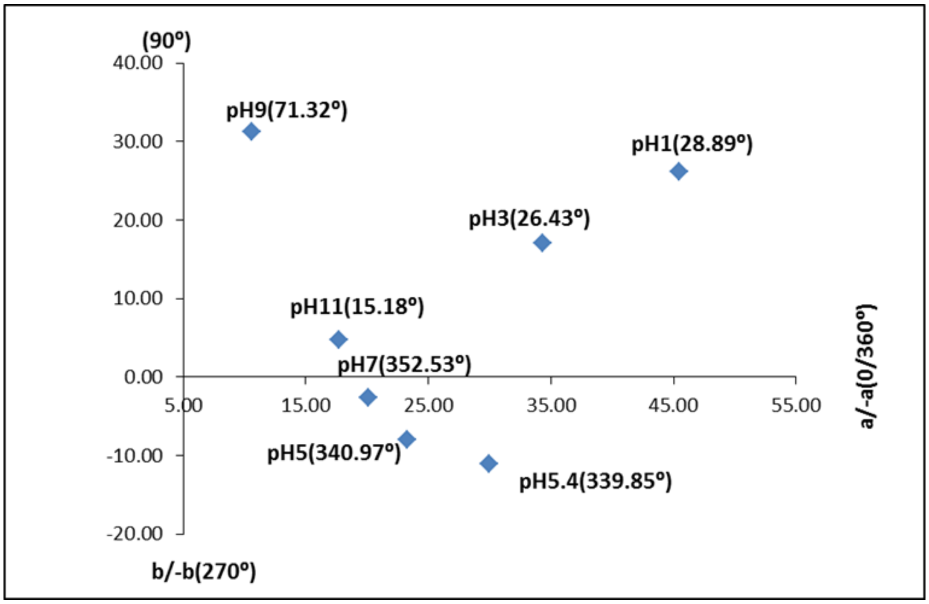
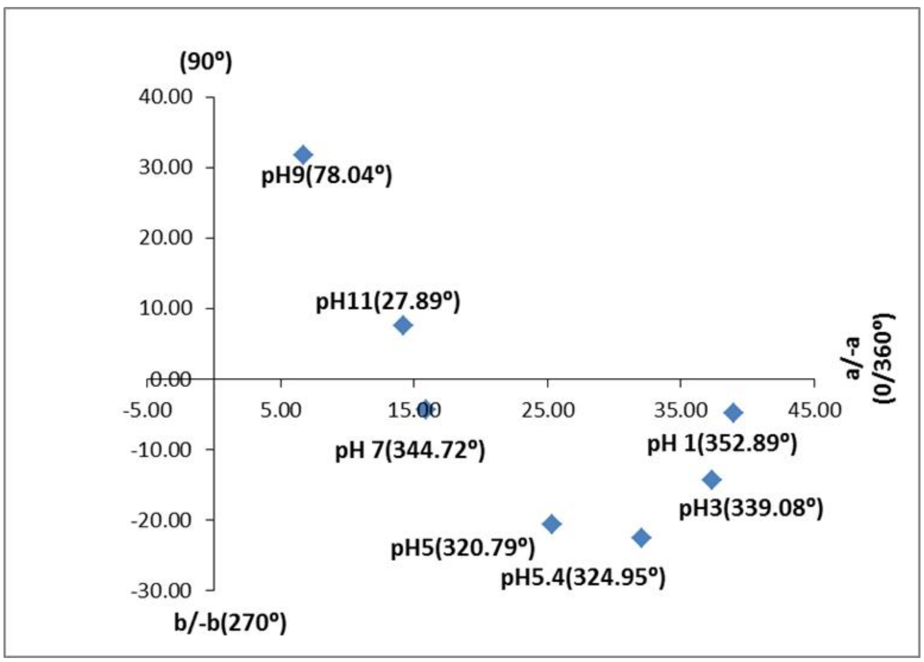
Furthermore, altered the pH for crude anthocyanin colourant containing 3% FA also affected the colour chromaticity. As seen in figure 4.8 above, the C* value for most acidic crude anthocyanin colourant pH1 (52.455±0.006) recorded as the highest chroma compared to the other sample at the beginning the storage.However, from the table It can be seen as starting pH 1 the chromaticity decrease (52.455±0.006) with increasing pH 11 (18.304±0.010) nevertheless when reaching pH 9 the chromaticity were slightly increase (32.953±0.009).On the hand, the chroma of the crude anthocyanin colourant also decreased during 3 month of storage which result in dull in colour. As seen in table, most coloured sample resulted for the sample at pH 3, the C* value recorded was (36.492±0.008) which gave in the highest C* value (brighter colour) compared to the other pH study. The most coloured sample at the beginning of the storage did not retained the colour stability which the colour of the samples strongly faded at the end of the storage (31.405±0.007) Same tendency was also obsereved for the origin sample without adjusted pH which the C* value are also decreased over the storage period, and showed better result compared to alkaline crude colourant pH 11 (11.47±0.010). Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

Hue, which also vital affect coused by variation of pH. As in table, Further augmentation of pH 1, H^o (29.890±0.0060) to higher pH 5.64 initiated an important counterclockwise shift of hue angle H^o (339.850±0.007), meaning that the hues now moved back to yellower tonalities. Based on figure 4.9(a), it shows that the pH 5.4 of *M. malabathricum* present in positive a* values (29.988±0.009) and negative b* values (-11.000±0.012) with hue angle (H^o (339.850±0.007)) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (45.478±0.007) and negative b* values moved to positive value (26.142±0.008) while hue angle moved clockwise to lower value (h_{ab} 29.890±0.006) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (10.556±0.009) and b* slightly increase to positive values (4.794±0.0060) while hue angle moved counterclockwise (h_{ab} 15.182±0.012).

Furthermore, the pH variation also affects the visual colour stability of crude anthocyanin colourat during storage.according to the figure 4.9(b) to 4.9(d) the highest colour enhancement was observed for the sample with pH 3 with the 1st month, figure 4.9(b) increased in hue angle to (339.079 ±0.0107) with a* positively increased (37.354±0.010) with positive b* value moved to negative b* value (-14.278±0.009) and slightly decreased h° in 2nd month(334.100±0.0103), figure 4.9(c) with a* positively increased (39.771±0.006) and negative b* value (-19.30833±0.005487). pH 3 retained the colours stability after three month of the storage,figure 4.9(d).Based on figure also, it clearly noticed that the visual colour of samples with more alkaline pH 11 easily faded during storage with the a* (1.472±0.007) moved to lower positive value and b* slightly moved to more yellower tonalities (11.373±0.007), while the hue angle moved counterclockwise (h_{ab} 82.621±0.0113) which showed the colour degradation of anthocyanin colourant containing 3% FA. Moreover, In contrast for crude colourant containing 3% FA, the most coloured sample at pH 1 in the beginning of the storage obviously experience calour degradation during 3 month of the storage compared to pH 5 and pH 5.4 with the a* values started to move to the less positive (17.340±0.004) and b* decreased to more positive value (11.376±0.007) with increasing the H^0 (56.487±0.008). Nonetheless it again can be noticed that, at the end of the storage, the pH 3 succesfully improved the colour stability of the crude colourant containing 35 FA which resulted in the brighter colour compared to the other pH study.

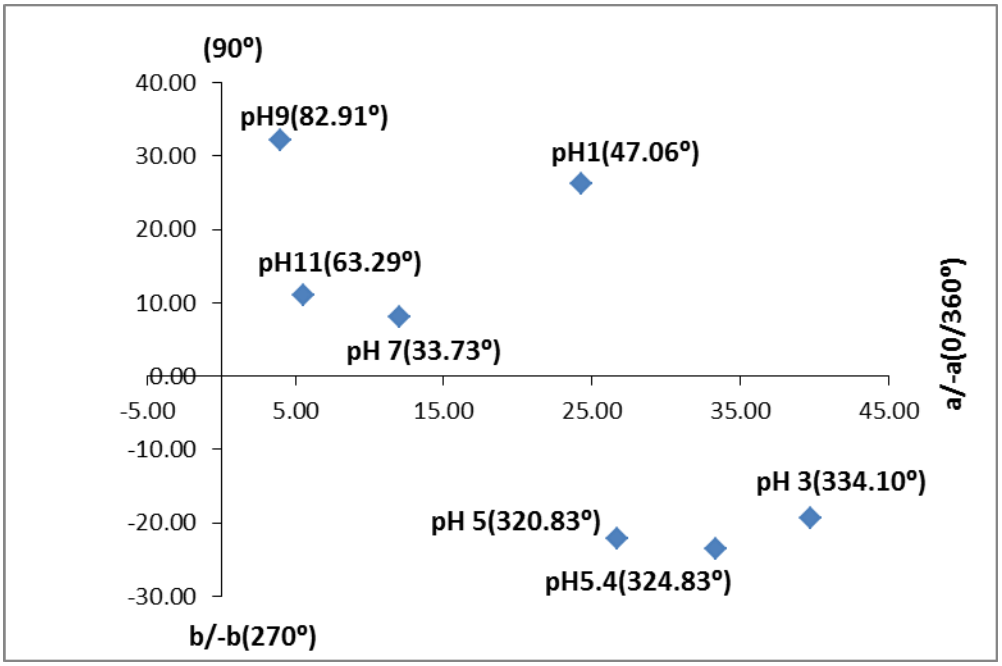


(a)



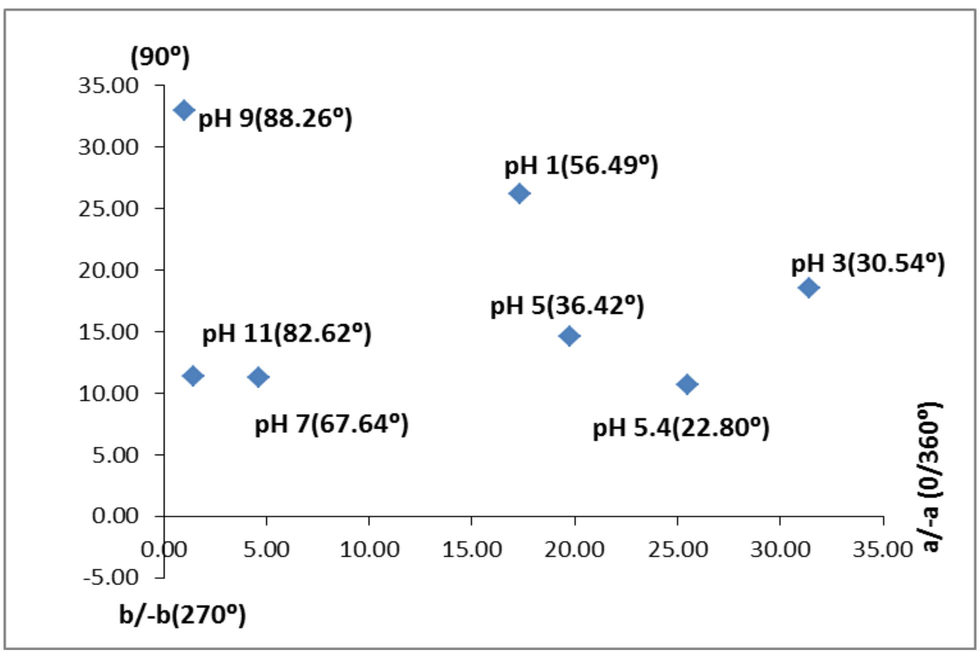
(b)

Figure 4.9: Relationship between pH variation and H◦ with a*b* co-ordinate for *M. malabathricum* containing 3% FA during (a) zero time of storage,(b) 1 month of storage,(c) 2month of storage and (c) 3 month of storage



(c)

'Table 4.9, continued'



(d)

'Table 4.9, continued'

Table 4.3: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin colourant from *M.malabathricum* containing 3% FA

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	$s_0=0.9767$	$s_1=0.7214$	$s_2=0.6104$	$s_3=0.4861$	$\Delta E_1=31.684$	$\Delta E_3=30.173$
pH 3	$s_0=0.7580$	$s_1=1.0327$	$s_2=1.1914$	$s_3=0.7003$	$\Delta E_1=33.578$	$\Delta E_3=3.639$
pH 5	$s_0=0.5173$	$s_1=0.8198$	$s_2=0.8896$	$s_3=0.5012$	$\Delta E_1=14.934$	$\Delta E_3=22.901$
pH 5.4	$s_0=0.6388$	$s_1=0.9262$	$s_2=0.9808$	$s_3=0.4941$	$\Delta E_1=13.884$	$\Delta E_3=22.940$
pH 7	$s_0=0.4239$	$s_1=0.3174$	$s_2=0.2605$	$s_3=0.2056$	$\Delta E_1=6.258$	$\Delta E_3=23.697$
pH 9	$s_0=0.7514$	$s_1=0.6736$	$s_2=0.6232$	$s_3=0.5007$	$\Delta E_1=5.873$	$\Delta E_3=24.006$
pH 11	$s_0=0.4291$	$s_1=0.3506$	$s_2=0.2159$	$s_3=0.1692$	$\Delta E_1=5.518$	$\Delta E_3=30.597$

Table 4.3 displays the Total Colour difference (ΔE) of crude anthocyanin colourant containing 3% FA with different pH (pH initial 5.4, pH 1, 3, 7, 9 and 11). Since the a* and b* parameters represent the redness and the yellowness on the chromaticity dimension, while the c* and h$^{\circ}$ parameter represent the Hunter a* and b* parameters and the ΔE represents the colour change of three colour coordinate (C*, L* and h) of sample before and after exposed to high UV irradiadiation for 3 month. Smallest (ΔE) was noticed for the samples with pH 11 $\Delta E_1=5.518$ at the beginning of the storage. After 3 month of the storage the $\Delta E_3=30.597$ was increased for pH 11 which showed the colour varied compared to the first month. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample at pH 3 ($\Delta E_1=33.578$) which showed that the crude anthocyanin colourant was successfully enhanced.however at the end of the storage pH 3

samples results in small colour changes (ΔE_3=3.639) but still most coloured than the others samples tested.

In addition, the saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. The results gained for this analysis of saturation displayed in table 6.7. According to the table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.4, pH 1, 3, 7, 9 and 11) for crude anthocyanin colourant containing 3% FA. From the table above the huge colour variation with the adjusting pH was observed. Highest colour saturation recorded for the samples at pH 3 (s_2=1.1914) in the 2^{nd} month of the storage and gradually decreased during storage with the saturation recorded were s_3=0.7003 which the highest saturation recorded compared to the other samples. Hence these results showed that pH 3 resulted in more saturated purple-blue colour compared to the other pH studied.However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.4291 to s_3S_3=0.1692 at the end of the storage. Therefore it directly noticed that, the colour of sample at pH 11 obviously lost the colour during the 3 month of the storage.

4.3. Colour Analysis of purified anthocyanin colourant from Fruit Pulp of M. Malabathricum

4.3.1. Influence of different percentage of FA added on Visual Colour Variation

Figure 4.10 present influence of different percentage of FA addition for purified fruit pulp of anthocyanin *M. Malabathricum* colourant on the values of the colour parameters (colorimetric indexes and CIELAB variables) in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness). Similar can be observed as crude anthocyanin colourant that at zero time strorage, the non-enhance crude anthocyanin colourant (0% FA) resulted in the lightest samples with highest L* (69.850 ± 0.011). However addition of FA (1%, 2%, 3%, 4% and 5% FA) significantly decreased the L* value and samples with the addition of 3% FA gaved the lowest L* value (55.309±0.008) followed by 2% FA added (60.942± 0.016). The lightness of the non-enhance crude anthocyanin colourant slightly increased upon storage under 100% lux intensity (17.55 lux), with the end of storage (3 month) the L* value recorded was (94.214 ±0.013) for non-enhance solution. These results revealed that the colour of non-enhance samples lighter after 3 month of storage compared to the samples containing FA. Furthermore the colour stability of purified anthocyanin improved with the addition of 3% FA with the lightness, L* of the sample decreased in 1 and 2 month of storage, whereas an insignificant increased in L* value was observed durng the last period of samples storage (62.570 ±0.010) meaning that colour of the 3% FA added also faded during the storage.

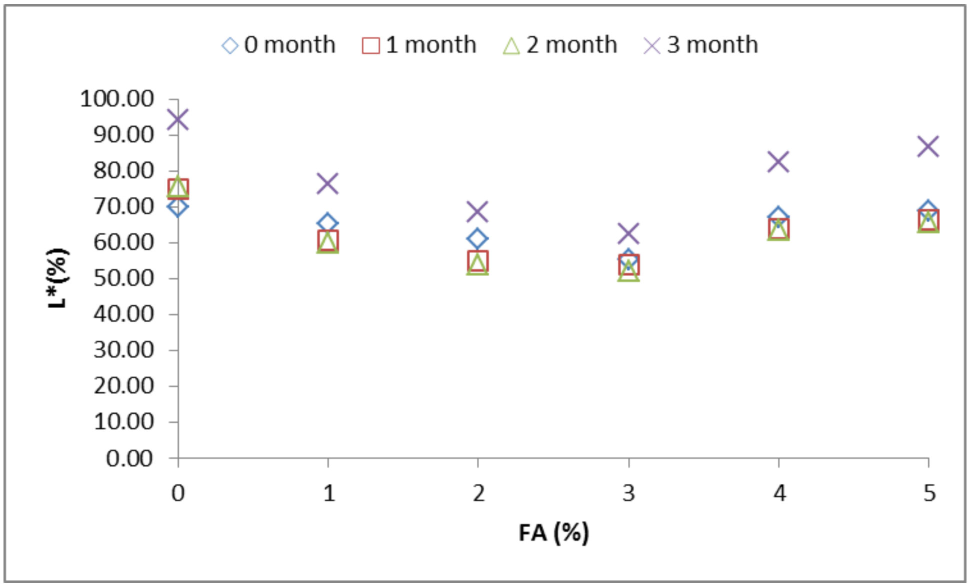


Figure 4.10: Relationship between FA percentage and L* values (%) for purified *M. malabathricum* anthocyanin colourant during 3 month of storage.

Furthermore, different addition of FA percentage also affects the colour chromaticity, C* values during 3 month of storage. Figure 4.11 shows the plot of graph C*(%) against percentage of FA added. It can be seen that, in the beginning of storage, zero time storage the C* of non-enhance samples resulted in dull colour with the lowest C* value (14.724±0.013). On the other hand, addition of FA positively augmented the C* value with resulted in brighter colour. According to the table below, crude anthocayanin colourant with the addition of 3% FA gave the brighter colour with highest C* value (31.053±0.007) compared to the other samples studied. Also, as seen in table further increased in FA % addition up to 4 and 5% FA resulted in decreased of C* value (17.776±0.018) and (17.380±0.014) respectively .The chroma results for purified anthocyanin colourant was observed to decreased over the storage period for non-enhance samples.Furthermore the C* for purified antghocyanin colourant with the addition of FA exhibit slightly increased upon storage up to 2 month before expericed decreased in C* at the end of the storage.This trend was obviously for 3% FA added crude colourant which the C* value increased over 2 month of storage (52.278±0.005), however extended the storage period up to 3 month resulted in significantly decreased in C* value (27.620 ± 0.004). The results gained for this investigation showed that 3% FA significantly enhance the colour of crude anthocyanin colourant by increased the C* value at the beginning of storage.Nevertheless, as non-enhance sample at the end of storage, the colour of 3% FA added samples also faded with resulted in decreased the C* value.On the other hand the end of stprage, 3% FA added still resulted in the highest C* (26.890±0.007) value which means more coloured compared to the others.

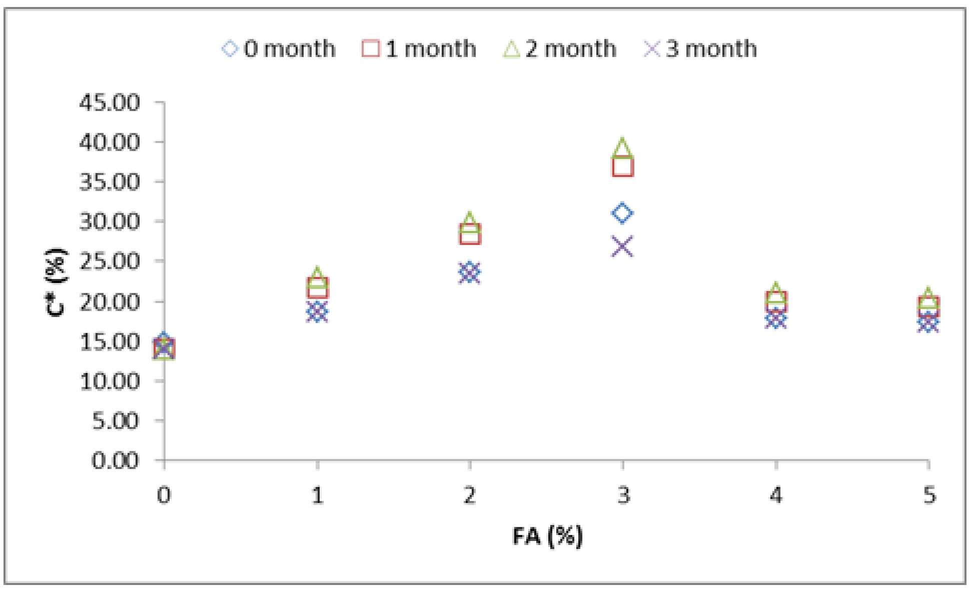
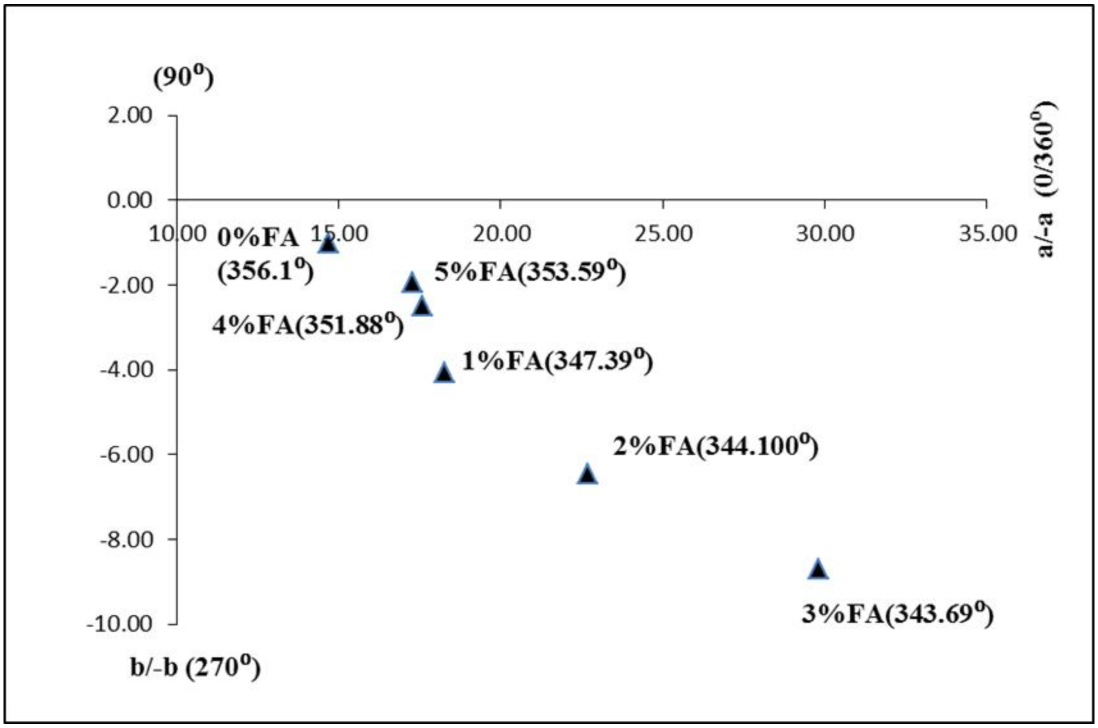


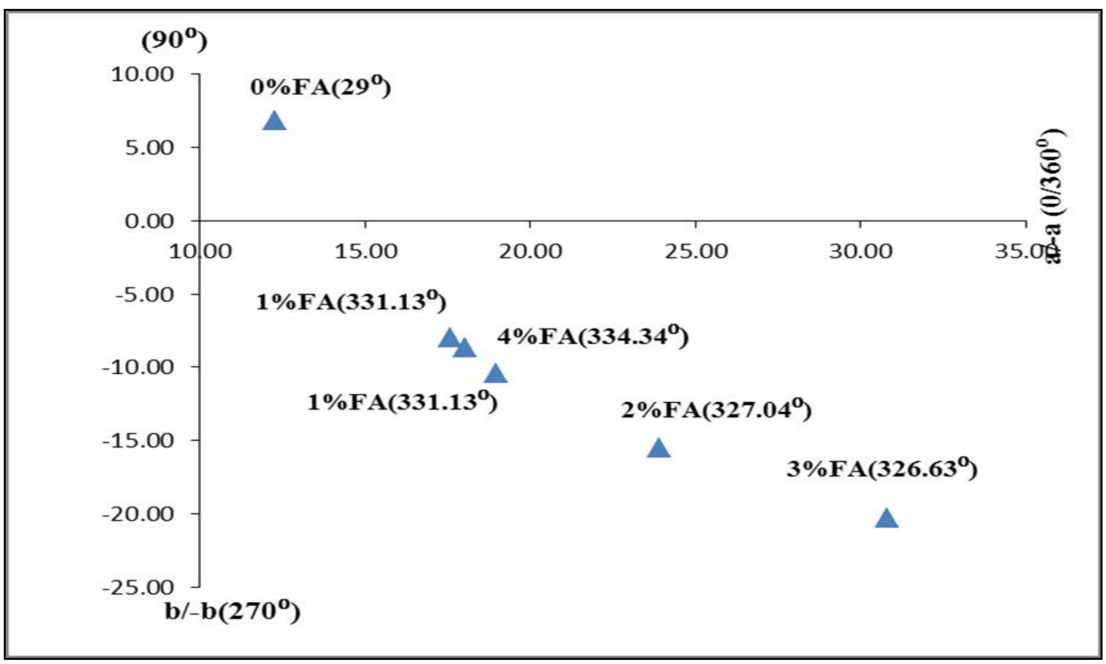
Figure 4.11: Relationship between FA percentage and C* values (%) for purified M. malabathricum anthocyanin colourant during 3 month of storage

Moreover, as seen in figure 4.12(a) to 4,12(d), hue angle value also change with the addition of FA.The initial colour position on the circle recorded for non-enhance purified anthocyanin colourant with the H^{o} (356.100±0.014), then the hue angle first moved to the lower value (counter clockwise) with the addition of 1% FA (347.390±0.014) to 3% FA (343.690±0.011). On the hand, based on the table below, it can visibly note that the non-enhanced sample for purified anthocyanin colourant from fruit pulp *M.malabathricum* present in positive a* value (14.690±0.015) and negative b* (-1.001±0.014) with hue angle (356.100±0.014) at zero time of storage. Though, addition of FA significantly enriched the blue colour, with resulted in more negative b* value since b* measures blueness when negative. it can be realized that addition of 3% FA gave better enhancement with resulted in positive a* value (29.805±0.011) and more negative b* value (-8.716±0.012) with the H^{o} (343.690±0.011).

Additionally, the colour of non-enhance sample (0 % FA) experience decreased in H^{o} to the lower value during 3 month of storage can be noted which means that the colours of the samples was faded. It can be realized that, at the end of storage, the a* value decreased to (11.001±0.017) which the lowest value and the b* increased to a more yellowness value (13.899±0.017) with the H^{o} (85.880±0.014).Nevertheless, the addition of 3% FA resulted in improved the colour stability of crude colourant by a* value recorded at the end of storage was highest(23.403±0.010) and b* lessly positive (13.243±0.009) with the H^{o} (29.504±0.005)

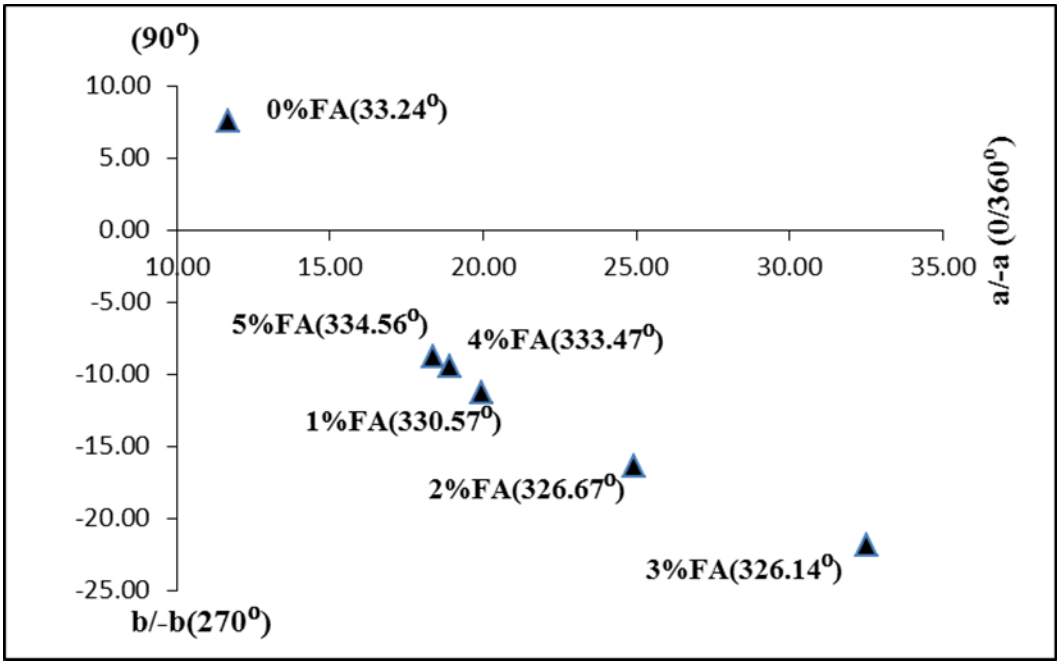


(a)



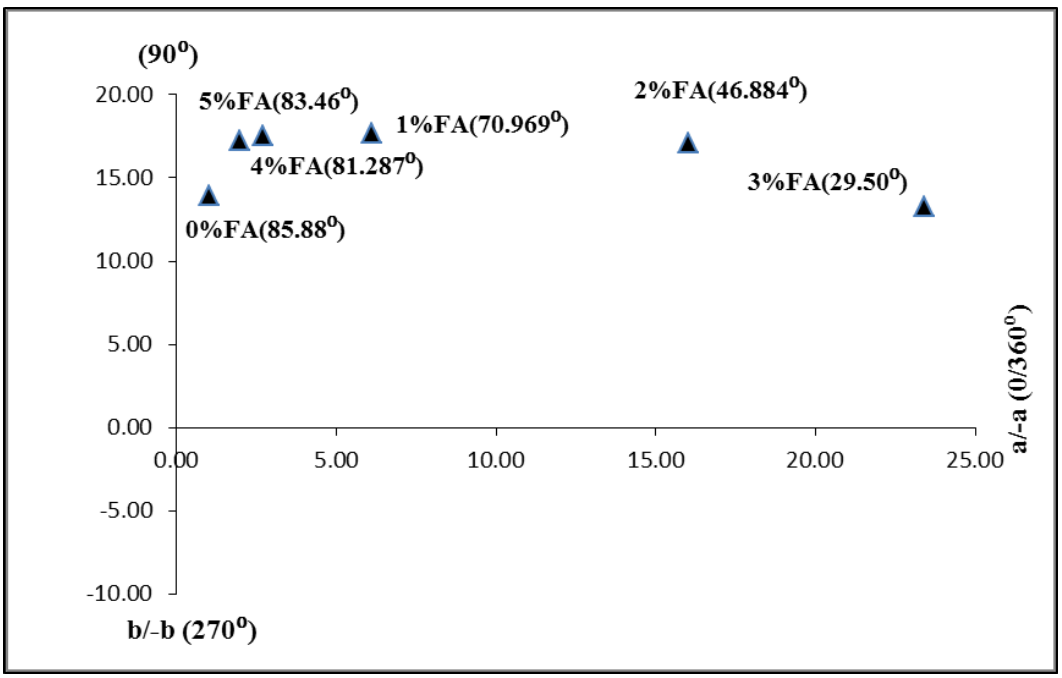
(b)

Figure 4.12:Relationship between percentage of FA and H◦ with a*b* co-ordinate for purified *M. malabathricum* anthocyanin colourant FA during (a) 0 month of storage, (b) 1 month,(c) 2 month and(d) 3month of storage



(c)

'Figure 4.12, continued'



(d)

'Figure 4.12, continued'

Table 4.4: Influence of different percentage of FA on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin colourant from *M.malabathricum*

FA(%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
0	s_0=0.2108	s_1=0.1873	s_2=0.1848	s_3=0.1479	ΔE_1=9.740	ΔE_3=31.670
1	s_0=0.2871	s_1=0.3570	s_2=0.3822	s_3=0.2448	ΔE_1=7.845	ΔE_3=27.342
2	s_0=0.3871	s_1=0.5196	s_2=0.5513	s_3=0.3421	ΔE_1=10.977	ΔE_3=25.611
3	s_0=0.5614	s_1=0.6863	s_2=0.7487	s_3=0.4298	ΔE_1=11.720	ΔE_3=23.998
4	S_0=0.2646	s_1=0.3121	s_2=0.3332	s_3=0.2152	ΔE_1=6.921	ΔE_3=29.366
5	s_0=0.2520	s_1=0.2917	s_2=0.3102	s_3=0.2000	ΔE_1=6.685	ΔE_3=30.379

The above result was further analysed in term of Total Colour difference (ΔE) and saturation(s).Table 4.4 displayed the result obtained from the analysis for purified anthocyanin colourant with different addition FA. The saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L.According to the table, the different percentage of FA gave variation of colour and resulted in different saturation of colour for purified anthocyanin colourant .Highest colour saturation recorded for the samples at 3% FA added (s_2=0.7487) in the 2nd month of the storage and gradually decreased during storage with the saturation recorded were s_3=0.4298 which the highest saturation recorded compared to the other samples. Therefore these results showed that samples containing 3% FA resulted in more vivid purple-blue colour compared to the other samples studied. However, as realized in the table the lowest saturation value recorded for the non-enhance samples with the saturation was observed to decreased over the storage period from s_0=0.2108, s_1=0.1873, s_2=0.1848 to s_3=0.1692 respectively. Therefore it directly noticed that, the visual colour of sample of non-enhance samples obviously bleached during the 3 month of the storage.

Furthermore, ΔE represents the colour change of three colour coordinate (C*, L* and h) of sample before and after exposed to high UV irradiation for 3 month. Smallest (**ΔE**) was noticed for the samples with 5% FA added ΔE_1=6.685 at the beginning of the storage. After 3 month of the storage the ΔE_3=30.379 was increased for which showed the colour of samples varied compared to the first month with the saturation only s_3=0.2000. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample with3% FA (ΔE_1=11.720) which showed that the purified anthocyanin colourant was effectively enhanced**.**however at the end of the storage ΔE of 3% FA samples was increased (ΔE_3=23.998) but still most coloured than the others samples tested.

4.3.2. Influence of different pH on Visual Colour Variation of purified anthocyanin colourant

Figure 4.13 present the influence of different pH (pH initial 5.7, pH 1,3, 7,9 and 11) on visual colour varion for purified fruit pulp of anthocyanin *M. Malabathricum* colourant in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness).It can be seen from pH 1 the lightness percentage of *Melastoma malabatricum* (69.294±0.018) decrease over increasing pH 11 (61.849±0.016) at zero time of storage. However, when approach pH 5.7, the lightness started to increase (69.850 ±0.011) before decrease when pH reaching pH 7 (66.707±0.010) and continue follow the decreasing trend.

The lightness of the samples was observed during the storage at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (97.853±0.017) after the 3 month of the storage which resulted in lighter colour. As expected, the slightly increased in L* value was observed for the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (83.997±0.014), (83.284±0.012) and (88.396±0.011) respectively.

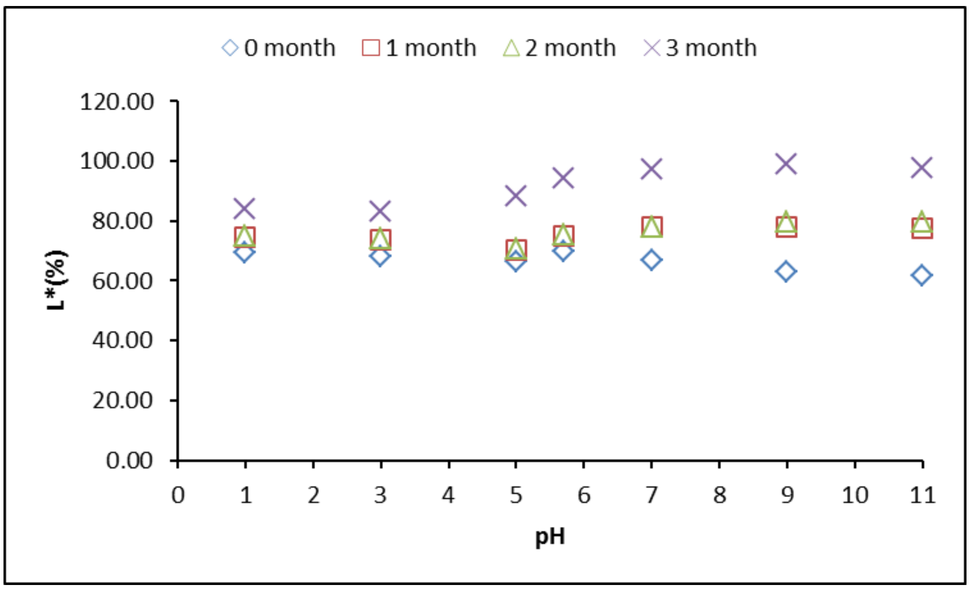


Figure 4.13: Relationship between pH variation and L* values (%) for purified *M. malabathricum* anthocyanin colourant during 3 month of storage

Furthermore, altered the pH for purified anthocyanin colourant also gave different colour chromaticity. According to figure 4.14 the C* value for most acidic crude anthocyanin colourant pH1 (44.954±0.015) noted as the highest chroma compared to the other sample. But, referring to the table It can be seen as starting pH 1 the chromaticity decrease

(44.954±0.015) with increasing pH 11 (13.397±0.018) nevertheless when reaching pH 9 the chromaticity were slightly increase (28.001±0.016).Besides, the Chroma of the purified anthocyanin colourant also decreased during 3 month of storage which result in dull in colour. As seen in figure 4.14, most intense sample pH 1 also experience the colour loss by dropping in C* value over time and it evidently at the end of the storage, the C* value recorded was (41.135±0.011) which still resulted in the highest C* value (brighter colour) compared to the other pH studied. Same tendency was also observed for the origin sample without adjusted pH (13.934±0.011) which the C* value are also decreased over the storage period, and showed better result compared to alkaline crude colourant pH 11(6.927±0.012). Consequently, these results point outs that, variation of pH significantly affect the C* value over the storage period.

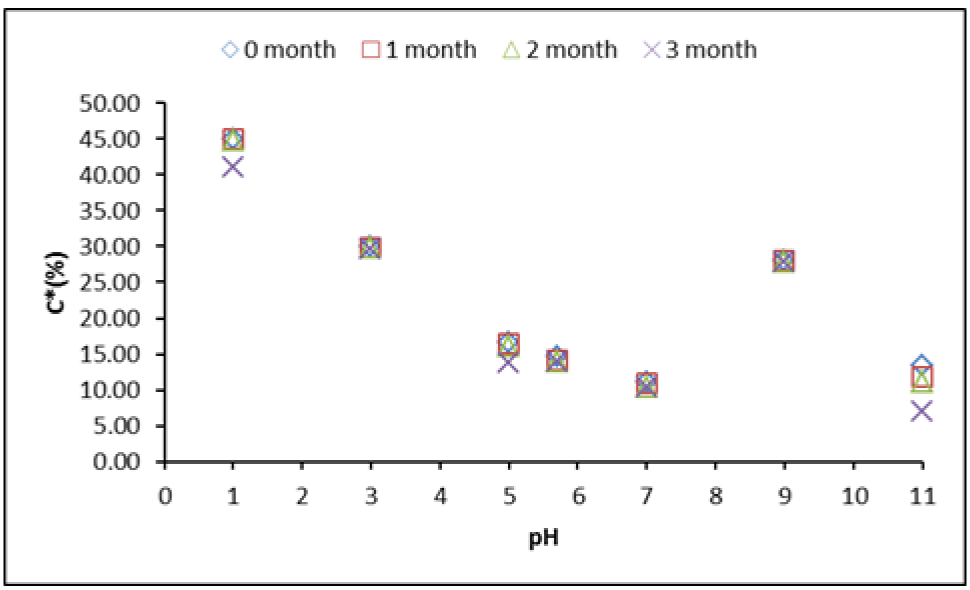
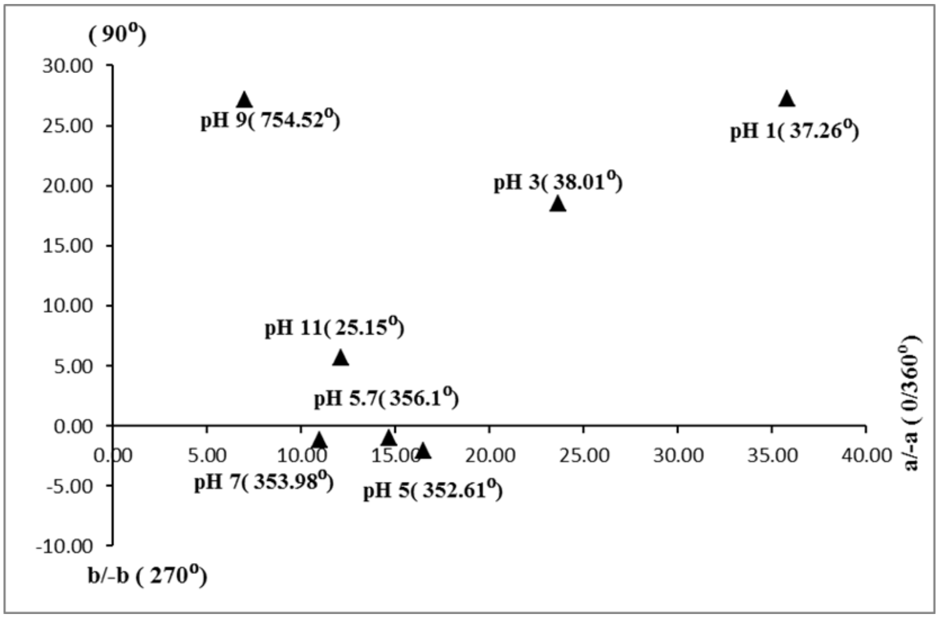


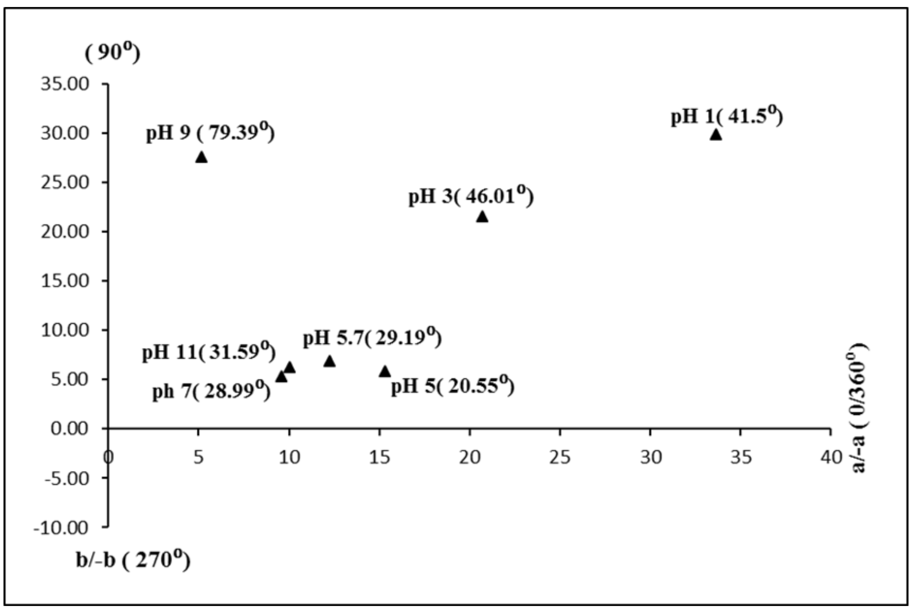
Figure 4.14: Relationship between pH variation and C* values (%) for purified *M. malabathricum* anthocyanin colourant during 3 month of storage

Variation of pH also affect the H^0,a* and b* parameter. Referring to figure 4.15(a) to 4.15(d), further rose of pH 1, H^o (37.258±0.013) to higher pH 5.7 initiated an essential counter clockwise shift of hue angle H^o (356.100±0.014), meaning that the hues now moved back to yellower tonalities. Based on figure 4.15(a), it shows that the pH 5.7 of *M.malabathricum* present in positive a* values (14.690±0.0155) and negative b* values (-1.001±0.014) with hue angle (h_{ab} 356.100±0.014) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (35.780±0.015) and negative b* values moved to positive value (27.216±0.010) while hue angle moved clockwise to lower value (h_{ab} 37.258±0.013) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (7.001±0.017) and b* slightly increase to positive values (27.112±0.014) while hue angle moved counterclockwise (h_{ab} 75.521±0.020).

Furthermore, the pH variation also affects the visual colour stability of purified anthocyanin colourat during storage. Based on the figure 4.15(b) to figure 4.15(d), it clearly noticed that the visual colour of samples with more alkaline pH easily faded during storage with the a* for pH 11(0.802±0.015) moved to lower positive value and b* slightly moved to more yellower tonalities (6.880±0.018), while the hue angle moved counterclockwise approach 90^o H^o(83.351±0.014) which showed the colour degradation of anthocyanin.Additionaly, figure 4.15(d) shows that the most coloured sample at pH 1 also underwent calour degradation during 3 month of the storage with the a* values started to move to the less positive(16.615±0.014) and b* increased to more positive value (37.631±0.012) with increasing the H^o (66.177±0.015). And it again can be noticed that, at the end of the storage, the pH 1 succesfully improved the colour stability of the crude colourant which resulted in the most coloured sample compared to the other pH study.

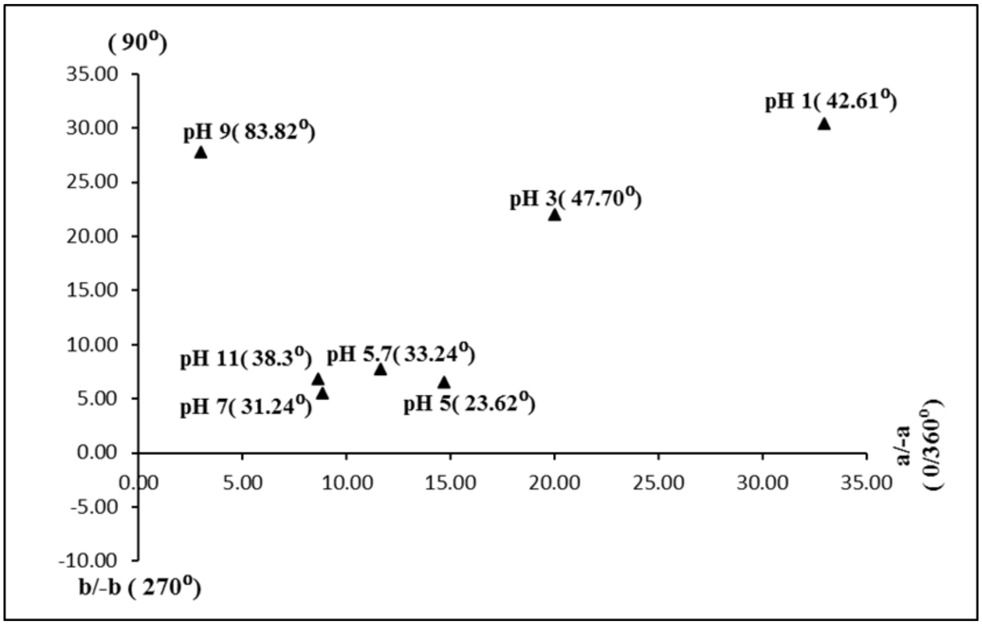


(a)



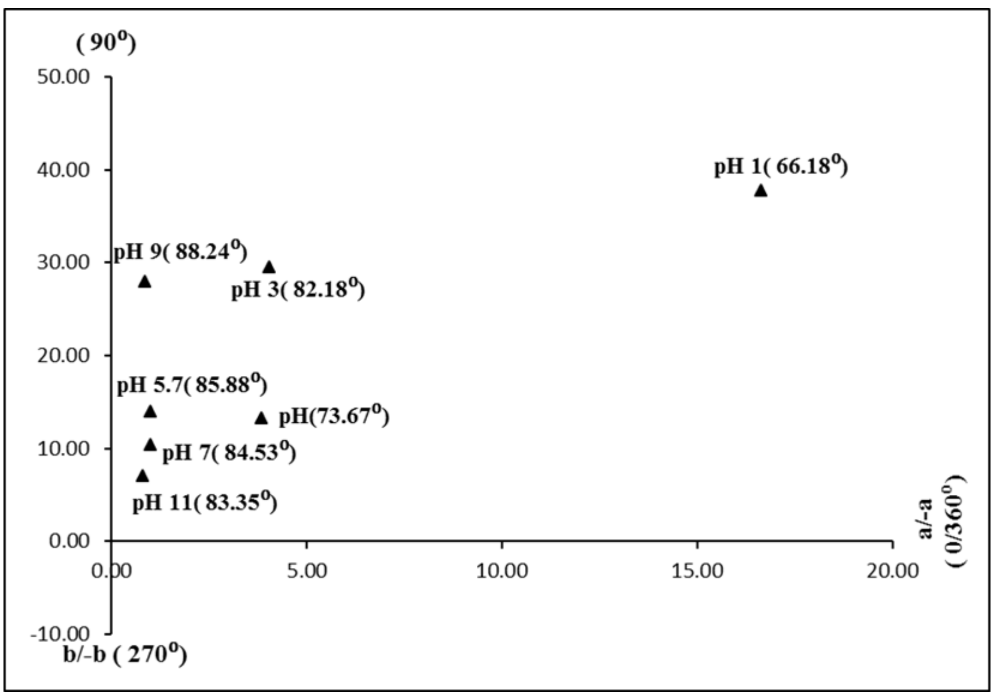
(b)

Figure 4.15: Relationship between pH variation and H◦ with a*b* co-ordinate for purified *M. malabathricum* during (a) 0 month of storage (b) 1 month of storage (c) 2 month of storage and (d) 3month of storage



(c)

'Figure 4.15, continued



(d)

'Figure 4.15, continued'

Table 4.5: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin colourant from *M.malabathricum*

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=0.6487	s_1=0.6023	s_2=0.5980	s_3=0.4897	ΔE_1=6.285	ΔE_3=26.232
pH 3	s_0=0.4411	s_1=0.4040	s_2=0.4014	s_3=0.3557	ΔE_1=7.194	ΔE_3=27.141
pH 5	s_0=0.2504	s_1=0.2325	s_2=0.2272	s_3=0.1548	ΔE_1=8.954	ΔE_3=29.678
pH 5.7	s_0=0.2108	s_1=0.1873	s_2=0.1848	s_3=0.1479	ΔE_1=9.740	ΔE_3=31.670
pH 7	s_0=0.1657	s_1=0.1407	s_2=0.1337	s_3=0.1064	ΔE_1=13.036	ΔE_3=34.164
pH 9	s_0=0.4455	s_1=0.3595	s_2=0.3496	s_3=0.2818	ΔE_1=15.097	ΔE_3=36.495
pH 11	s_0=0.2166	s_1=0.1517	s_2=0.1382	s_3=0.0708	ΔE_1=15.935	ΔE_3=37.762

Total Colour difference (ΔE) of purified anthocyanin colourant with different pH (pH initial 5.7, pH 1, 3, 7, 9 and 11) display in table 4.5 which the ΔE represents the colour change of three colour coordinate (C*, L* and h) of sample before and after exposed to high UV irradiation for 3 month. Smallest (ΔE) was noticed for the samples with pH 1 ΔE_1=6.285 at 1st month of the storage. After 3 month of the storage the ΔE_3=26.232was increased for pH 1 which showed the colour change compared to the first month. Conferring to the table, it can clearly noticed that the highest ΔE at the first month recorded for the sample at pH 11 (ΔE_1=15.935) and at the end of the storage pH 11 gave the highest total color change compared to the other samples tested (ΔE_3=37.762) which showed the huge colour different.

The saturation (s) displays in table above. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value,s. As observed in table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.7, pH 1, 3, 7, 9 and 11). Highest colour saturation recorded for the samples at pH 1 (s_0=0.6487) and gradually decreased during storage with the saturation recorded were s_1=0.6023, s_2=0.5980, s_3=0.4897 correspondingly which showed that pH 1 resulted in more saturated red colour compared to the other acdic pH. Nevertheless the decreasing of the saturation over 3 month of storage period showed that colour of pH 1 samples also degraded.However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.2166 to s_3=0.0708 at the end of the storage. Therefore it directly perceived that, the colour of sample at pH 11 obviously colourless during the 3 month of the storage.

4.3.3. Influence of different pH on Visual Colour Variation of purified anthocyanin colourant containing 3% FA

Influence of different pH (pH initial 5.5, pH 1, 3, 7, 9 and 11) on visual colour variation for purified fruit pulp of anthocyanin *M. malabathricum* colourant containing 3% FA displays in Figure 4.16. Parameter in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) was studied.It can be seen that the lightness percentage decreased with increased in pH from pH 1 (59.442±0.011) to pH 11 (50.432±0.008) at zero time of storage. On the other hand, when approach pH 5.5 the lightness started to slightly increase (55.309±0.008) before continued decreased when pH approach pH 7 (51.678±0.009). According to the table, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (77.319±0.010) after the 3 month of the storage. As expected, the slightly increased in L* value was observed for the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (70.444±0.011), (58.378±0.008) and (55.241±0.012) respectively. Based on the results gained, the colour stability of the crude anthocyanin colourant degraded during the storage period under UVB- irradiation by increasing the the L* value which was resulted in lighter colour and the end of the storage pH 3 presents the most brighter sample with L* value (58.378±0.008)

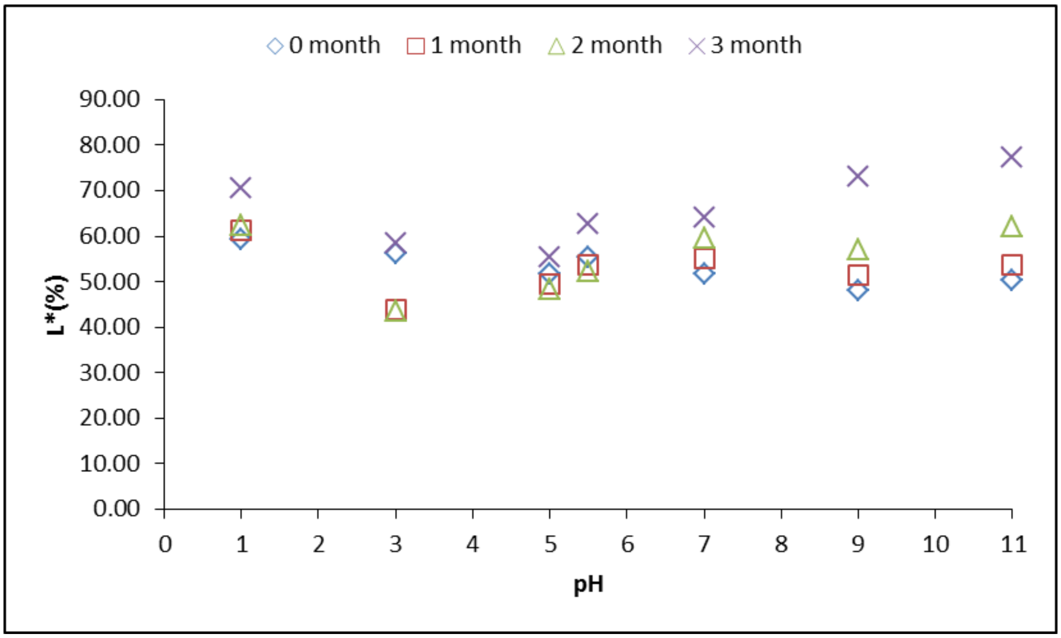


Figure 4.16: Relationship between pH variation and L* values (%) for purified *M. malabathricum* anthocyanin colourant containing 3% FA during 3 month of storage.

Besides, altered the pH for purified anthocyanin colourant containing 3% FA also affected the colour chromaticity. As seen in figure 4.17, the C* value for most acidic crude anthocyanin colourant pH1 (50.086±0.009) recorded as the highest chroma compared to the other sample at the beginning the storage.However, from the table It can be seen as starting pH 1 the chromaticity decrease (50.086±0.009) with increasing pH 11 (16.185±0.008) which showed the huge different in C* nevertheless when reaching pH 9 the chromaticity were slightly increase (27.597±0.007).On the hand, the chroma of the purified anthocyanin colourant also decreased during 3 month of storage which result in dull in colour. As observed in table, most coloured sample resulted for the sample at pH 3, the C* value recorded was (35.547±0.009) which gave in the highest C* value (brighter colour) at the end of storage (34.834±0.006) compared to the other pH study. The most coloured sample at the beginning of the storage did not retained the colour stability which the colour of the samples strongly faded at the end of the storage (29.252±0.007).Similar trend was also observed for the origin sample without adjusted pH which the C* value are also decreased over the storage period, and showed better result compared to alkaline crude colourant pH 11 (12.132 ±0.006). Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

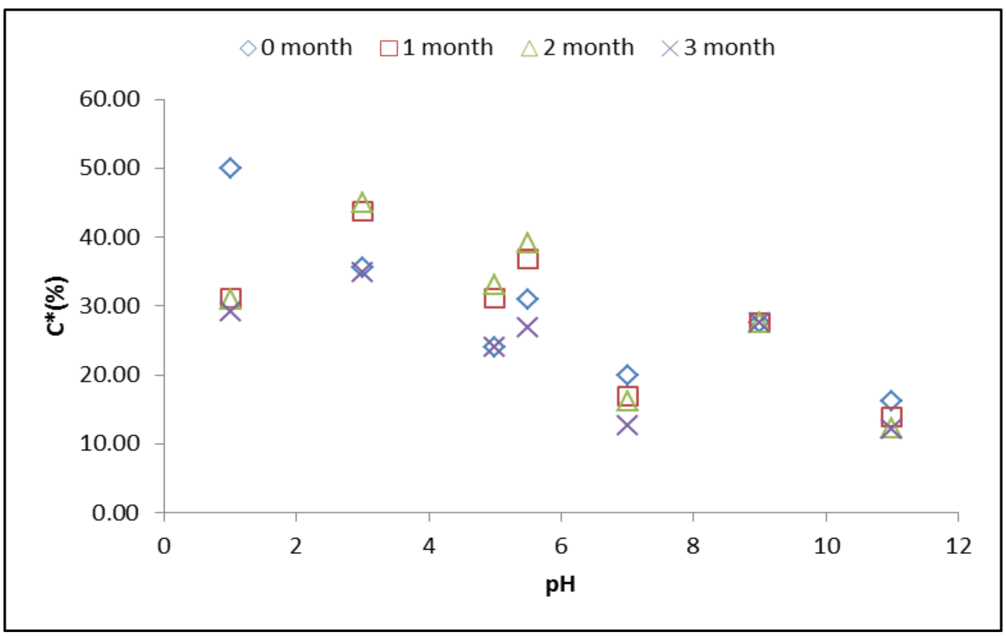
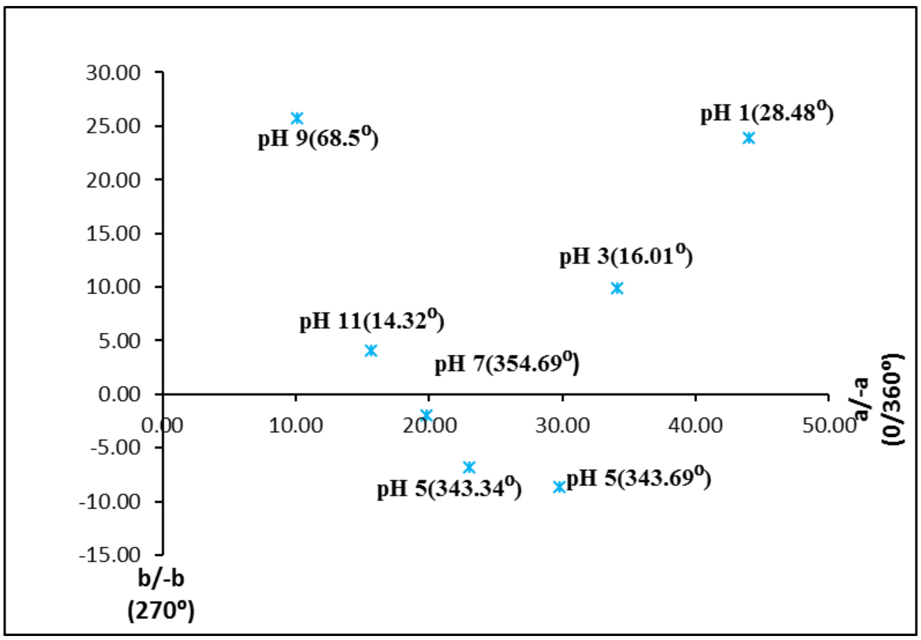


Figure 4.17: Relationship between pH variation and L* values (%) for purified *M. malabathricum* anthocyanin colourant containing 3% FA during 3 month of storage

Furthermore, as displays in figure 4.18(a) to 4.18(d0, Further adjusted of pH 1, H^o (28.476±0.009) to higher pH 5.64 initiated an important counterclockwise shift of hue angle H^o (343.690±0.011), meaning that the hues now moved back to yellower tonalities. It shows that the pH 5.5 of *Melastoma malabathricum* present in positive a* values (44.027±0.013) and negative b* values (-8.716±0.012) with hue angle H^o (343.690±0.011) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (44.027±0.013) and negative b* values moved to positive value (23.881±0.012) while hue angle moved clockwise to lower value (28.476±0.009) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (10.114±0.005) and b* slightly increase to positive values (25.677±0.007) while hue angle moved counterclockwise (68.500±0.010).

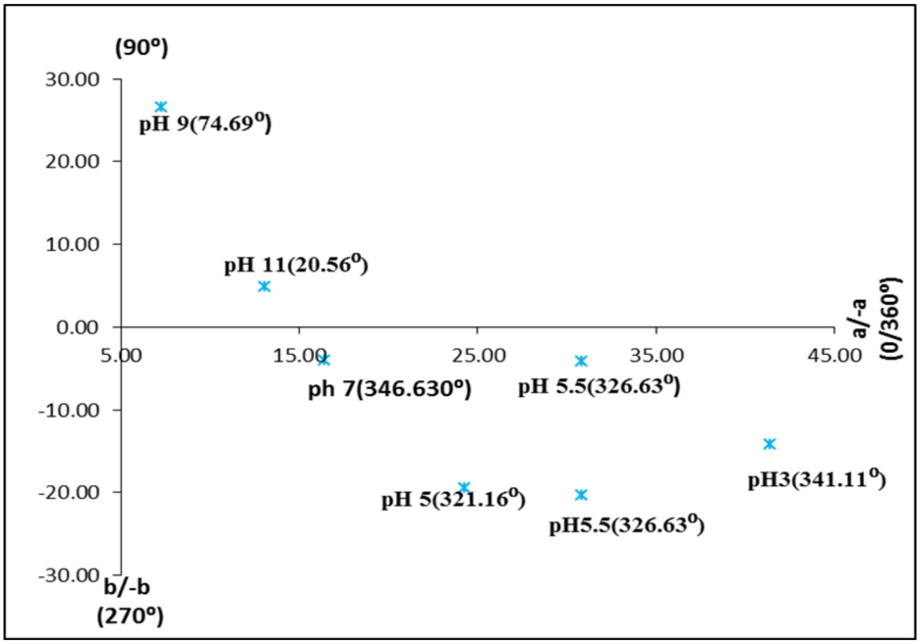
pH variation also affects the visual colour stability of crude anthocyanin colourat during storage. which the highest colour enhancement was observed from the table for the sample with pH 3 with the 1st month increased in hue angle to (341.110±0.011) with a* positively increased (23.048±0.012) with positive b* value moved to negative b* value(-6.897±0.007) and slightly decreased h° in 2^{nd} month (340.310±0.010) with a* positively increased (42.367±0.008) and negative b* value (-15.157±0.009). pH 3 retained the colours stability after three month of the storage. Based on figure 4.18(d), it clearly noticed that the visual colour of samples with more alkaline pH 11 easily faded during storage with the a* (32.032±0.006) moved to lower positive value and b* slightly moved to more yellower tonalities (13.690±0.007), while the hue angle moved counterclockwise H^0 (23.14±0.012) which showed the colour degradation of anthocyanin colourant containing 3% FA.

Moreover, In contrast for crude colourant containing 3% FA, the most coloured sample at pH 1 in the beginning of the storage obviously experience calour degradation during 3 month of the storage compared to pH 5 and pH 5.4 with the a* values started to move to the less positive (15.362±0.008) and b* decreased to more positive value (24.894±0.006) with increasing the H^0 (58.321±0.013). Nonetheless it again can be noticed that, at the end of the storage, the pH 3 succesfully improved the colour stability of the crude colourant containing 3FA which resulted in the most brighter colour compared to the other pH study.



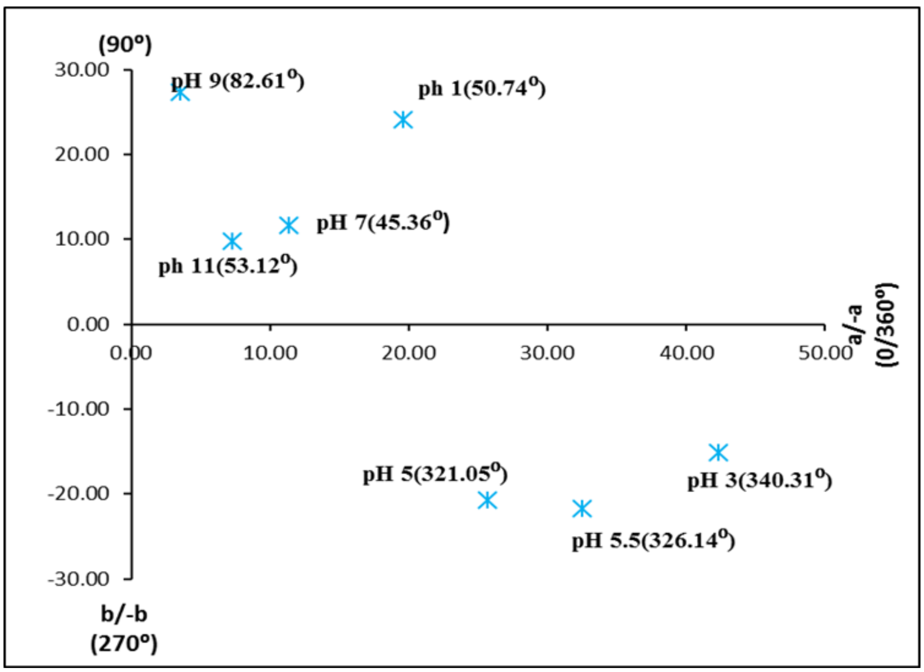
(a)

Figure 4.18: Relationship between pH variation and H◦ with a*b* co-ordinate for *purified M. malabathricum* containing 3%FA during (a) 0 month,(b) 1 month, (c) 2 month and (d) 3 month of storage



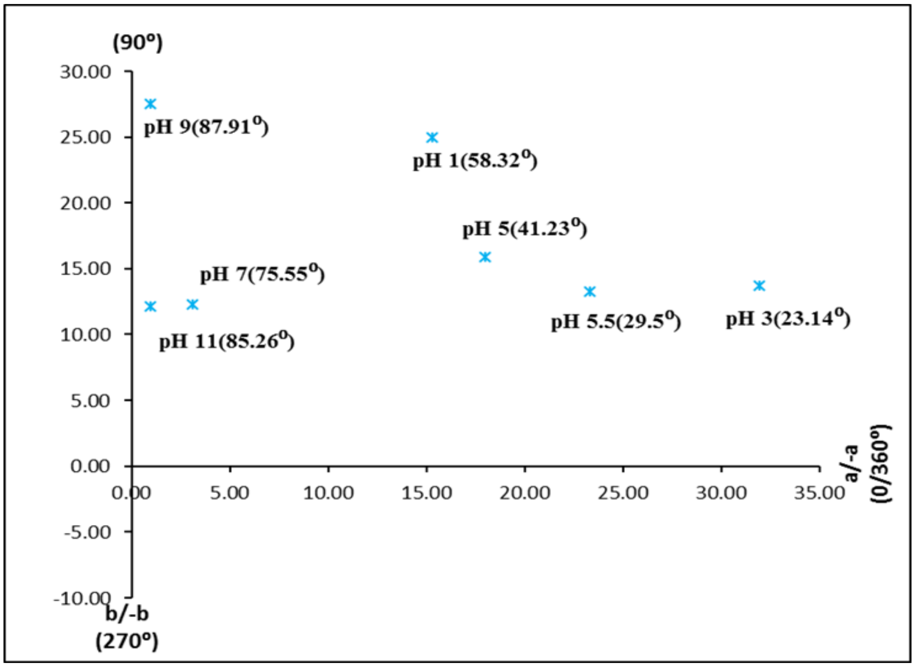
(b)

'Figure 4.18, continued'



(c)

'Figure 4.18, continued'



(d)

'Figure 4.18, continued'

Table 4.6: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin colourant from *M.malabathricum* containing 3 % FA

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=0.8426	s_1=0.5072	s_2=0.4971	s_3=0.4152	ΔE_1=31.009	ΔE_3=30.720
pH 3	s_0=0.6338	s_1=0.9956	s_2=1.0297	s_3=0.5967	ΔE_1=27.820	ΔE_3=4.992
pH 5	s_0=0.4640	s_1=0.6281	s_2=0.6844	s_3=0.4350	ΔE_1=12.882	ΔE_3=23.524
pH 5.5	s_0=0.5614	s_1=0.6863	s_2=0.7487	s_3=0.4298	ΔE_1=11.720	ΔE_3=23.998
pH 7	s_0=0.3863	s_1=0.3063	s_2=0.2730	s_3=0.1966	ΔE_1=5.433	ΔE_3=25.267
pH 9	s_0=0.5746	s_1=0.5370	s_2=0.4813	s_3=0.3770	ΔE_1=4.472	ΔE_3=26.614
pH 11	s_0=0.3209	s_1=0.2602	s_2=0.1965	s_3=0.1569	ΔE_1=4.227	ΔE_3=31.683

Table 4.6 displays the Total Colour difference (ΔE) of purified anthocyanin colourant containing 3% FA with different pH (pH initial 5.5, pH 1, 3, 7, 9 and 11).Smallest (ΔE) was noticed for the samples with pH 11 ΔE_1=4.227at the beginning of the storage. After 3 month of the storage the ΔE_3=31.683was increased for pH 11 which showed the losses compared to the first month. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample at pH 1 (ΔE_1=31.009) which showed that the crude anthocyanin colourant was successfully enhanced**.** However at the end of the storage pH 1 samples results in small colour changes (ΔE_3=30.720).

In addition, the saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. The results gained for this analysis of saturation displayed in table 4.6. According to the table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.5, pH 1, 3, 7, 9 and 11) for purified anthocyanin colourant containing 3% FA. From the table above the huge colour variation with the adjusting pH was observed. Highest colour saturation recorded for the 1st month for samples at pH 3 (s_1=0.9956) and continued to increase in the 2nd month (s_2=1.0297) of the storage and gradually decreased during storage with the saturation recorded were s_3=0.5967 which the highest saturation recorded compared to the other samples. Hence these results showed that pH 3 resulted in more saturated purple-blue colour compared to the other pH studied. However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.3209 to s_3=0.1569 at the end of the storage.

Therefore it directly noticed that, the colour of sample at pH 11 obviously lost the colour during the 3 month of the storage.

CHAPTER 5: RESULTS OF CIE COLOUR ANALYSIS SYSTEM FOR ANTHOCYANIN- PVA BLENDS

5.1. Introduction

Chapter 5 gives detailed investigation of stability and colour analysis study of the crude and purified anthocyanin colourant from *M. malabathricum* blend with PVA for coating system. This chapter begins with the colour variation of crude and purifiied colourant of *M. Malabathricum*-PVA blends during storage under UVB – irradiation (100% lux intensity) for 93 days of storage in order to study effect addition of Ferulic Acid (FA) as stabiliser on colour visual variation by using CIELAB colour analysis. And effect of pH to the crude and purified anthocyanin-PVA blend also studied. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences). Multifactor analysis of variance was applied with source of variance and color measurement instruments. Differences between means were tested using analysis of variance (ANOVA) with level significant of $P<0.05$. The statistical methods used for the data analysis were two-way analysis of variance (ANOVA) to find out whether there is a relationship between percentage of FA and pH variation on visual colour variation

5.2. Colour Analysis of crude anthocyanin-PVA blends from Fruit Pulp of M. Malabathricum

5.2.1. Influence of different percentage of FA added on Visual Colour Variation

Influence of different percentage of FA addition for crude fruit pulp of anthocyanin *M. Malabathricum*-PVA blends on the values of the colour parameters (colorimetric indexes and CIELAB variables) in terms of L* (lightness), C* (chroma), H˚ (hue angle notation

h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) present in table 5.2. Similar tendency can be obsereved as crude anthocyanin colourant that at zero time strorage, the non-enhance crude fruit pulp of anthocyanin *M. Malabathricum*-PVA blends (0% FA) resulted in the lightest samples with highest L* (63.813±0.0187),as seen in figure 5.1. However addition of FA (1%, 2%, 3%, 4% and 5% FA) significantly decreased the L* value and samples with the addition of 3% FA gaved the lowest L* value (49.144±0.0078) followed by 2% FA added (54.910±0.0089). The lightness of the non-enhance crude fruit pulp of anthocyanin *M. Malabathricum*-PVA blends slightly increased upon storage under 100% lux intensity (17.55 lux), with the end of storage (3 month) the L* value recorded was (78.704±0.0099) for non-enhance samples. These results inffered that the colour of non-enhance samples lighter after 3 month of storage compared to the samples containing FA. Furthermore the colour stability of crude anthocyanin-PVA blends improved with the addition of 3% FA with the lightness, L* of the sample decreased in 1 and 2 month of storage, however increased in L* value was observed during the last period of samples storage (50.997±0.0113) meaning that colour of the 3% FA added slighly faded during 3 month of storage.

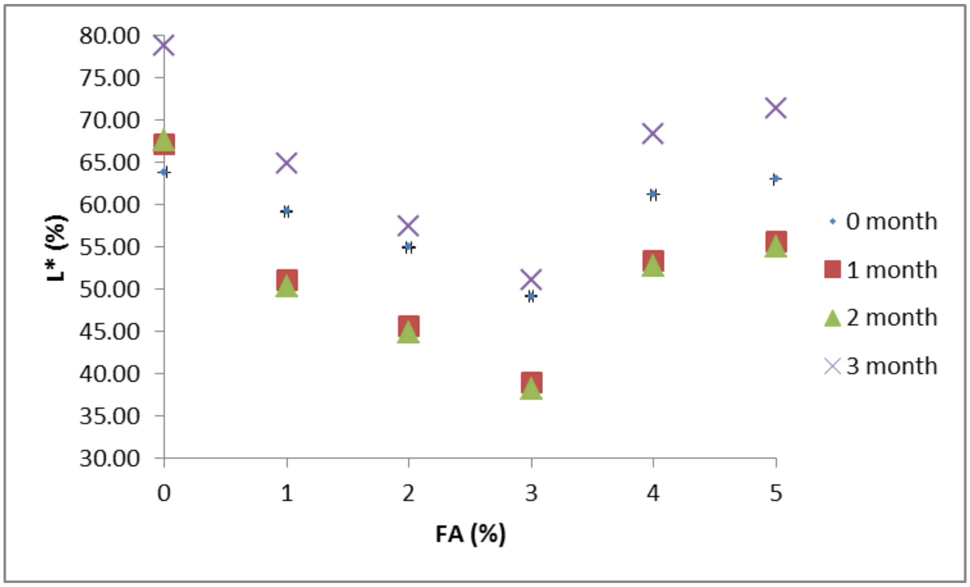


Figure 5.1: Relationship between FA percentage and L* values (%) for *M. malabathricum*-PVA blends during 3 month of storage

Different addition of FA percentage also affects the colour chromaticity, C* values during 3 month of storage. At zero time storage, as shows in figure 5.2, the C* of non-enhance samples resulted in dull colour with the lowest C* value (17.962±0.0118). On the other hand, addition of FA positively augmented the C* value with resulted in brighter colour. According to this figure also, crude anthocayanin-PVA blends with the addition of 3% FA gave the brighter colour with highest C* value (31.349±0.007) compared to the other samples studied. Also, as seen in table further increased in FA % addition up to 4 and 5% FA resulted in decreased of C* value (20.579±0.0176) and (19.047±0.0153) respectively .The chroma results for crude anthocyanin-PVA blends was observed to decreased over the storage period for non-enhance samples.Additionally the C* for crude anthocyanin-PVA blends with the addition of FA exhibit slightly increased upon storage up to 2 month before expericed decreased in C* at the end of the storage.This trend was obviously for 3% FA added crude colourant which the C* value increased over 2 month of storage

(43.368±0.0102), though, extended the storage period up to 3 month resulted in significantly decreased in C* value (19.836±0.012). The results gained for this investigation showed that 3% FA significantly enhance the colour of crude anthocyanin PVA blends by increased the C* value at the beginning of storage.Nevertheless, as non-enhance sample at the end of storage, the colour of 3% FA added samples also faded with resulted in decreased the C* value.On the other hand, at the end of storage, 3% FA added still resulted in the highest C* (19.836±0.0124) value which means more coloured compared to the others.

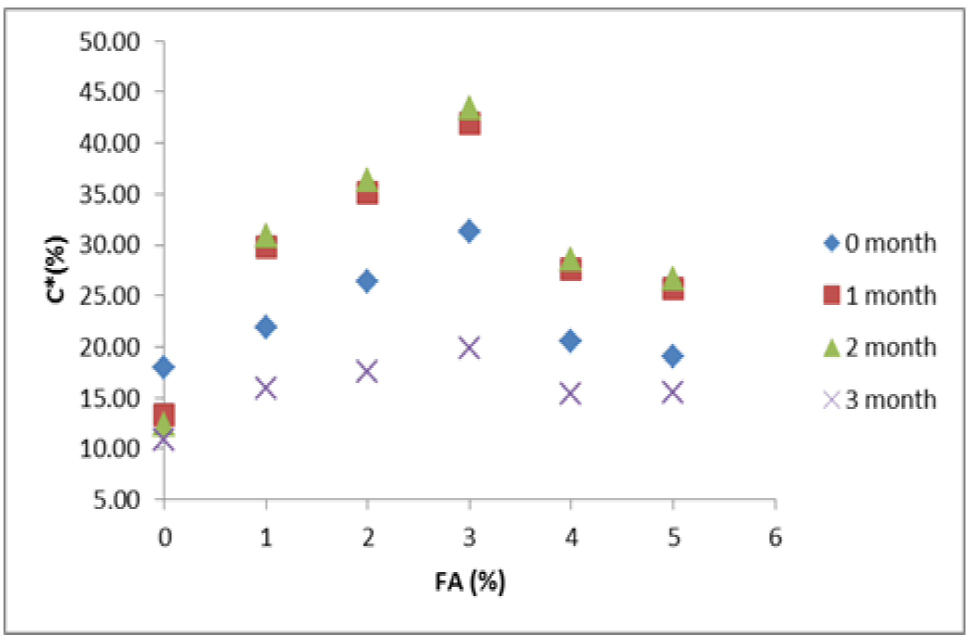
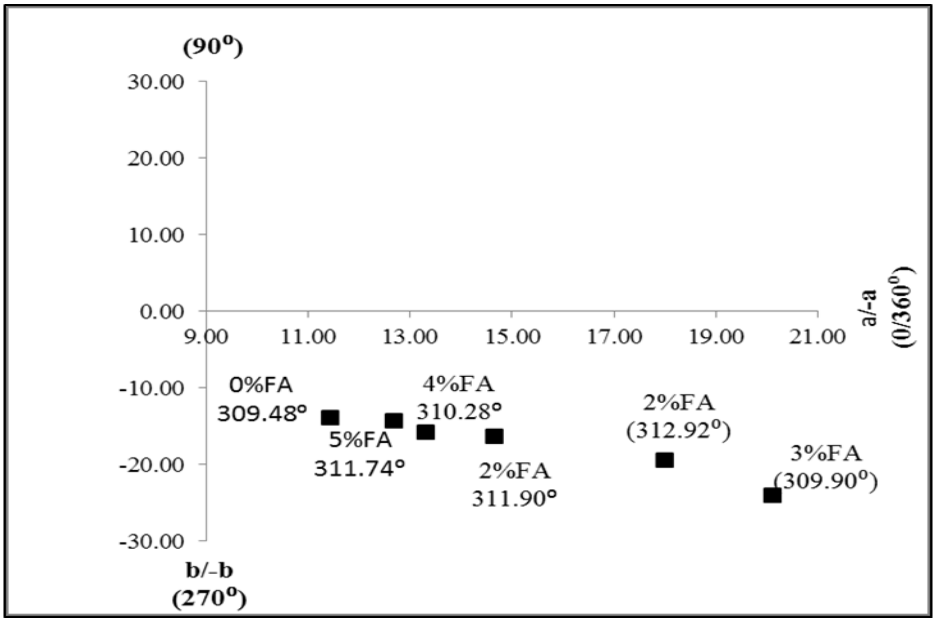


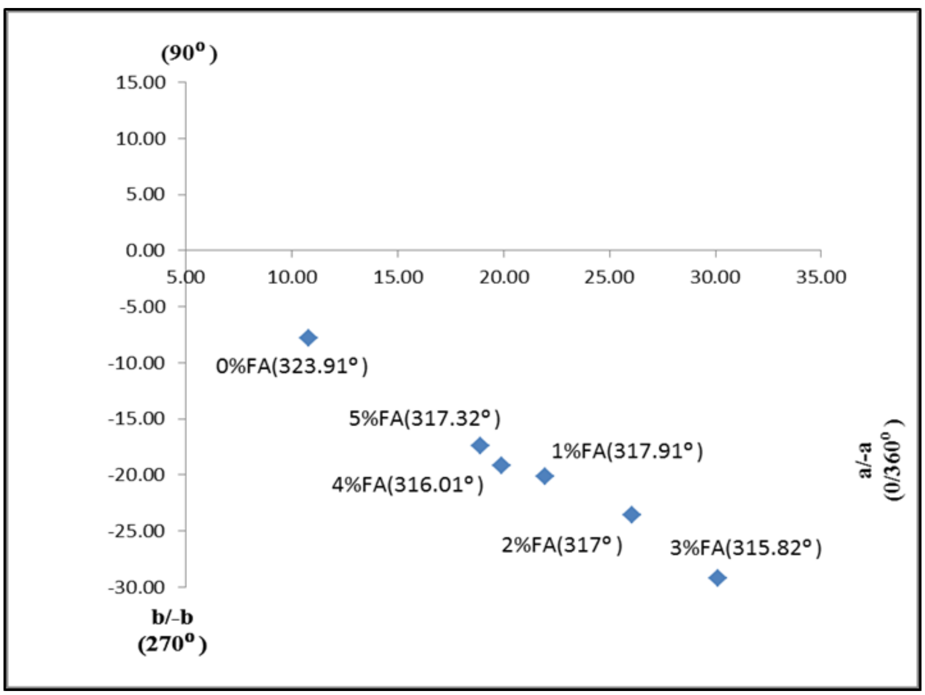
Figure 5.2: Relationship between FA percentage and C* values (%) for *M. malabathricum*-PVA blends during 3 month of storage

As seen in figure 5.3(a) to figure 5.3(d), hue angle also affected with the addition of FA.The initial colour position on the circle recorded for non-enhance crude anthocyanin-PVA blends with the H° (309.479±0.0113), then the hue angle first moved counter clockwise with the addition of 1% FA (311.90±0.0153) and clockwise to 3% FA (309.900±0.0083). On the hand, based on the figure 5.3(a), it can visibly note that the non-enhanced sample for crude anthocyanin-PVA blends from fruit pulp *M.malabathricum* present in positive a* value (11.421±0.0141) and negative b* (-13.865±0.0173) with hue angle (309.479±0.011) at zero time of storage. However, addition of FA significantly enriched the blue colour, with resulted in more negative b* value since b* measures blueness when negative. According to the results obtained, it can be realized that addition of 3% FA gave better enhancement with resulted in positive a* value (20.1106±0.0124) and more negative b* value (-24.050±.0086) with the H° (309.900±0.0083).

Moreover, the colour of non-enhance sample (0 % FA) experience decreased in H° to the lower value during 3 month of storage, as displayed in figure 5.3(b) to 5.3(d), which means that the colours of the samples was fadded. It can be realized that, at the end of storage, figure 5.3(d), the a* value decreased to (4.221±0.0182) which the lowest value and the b* increased to more yellowness value (2.1366±0.0072) with the H° (67.139±0.0153).Nevertheless, addition of 3% FA resulted in improved the colour stability of crude anthocyanin-PVA blends by a* value recorded at the end of storage was highest (19.721±0.007) and b* still in negative value (2.136±0.007) with the H° (353.810±0.008). The detailed results for colour analysis study for anthocyanin-PVA listed in table 5.2

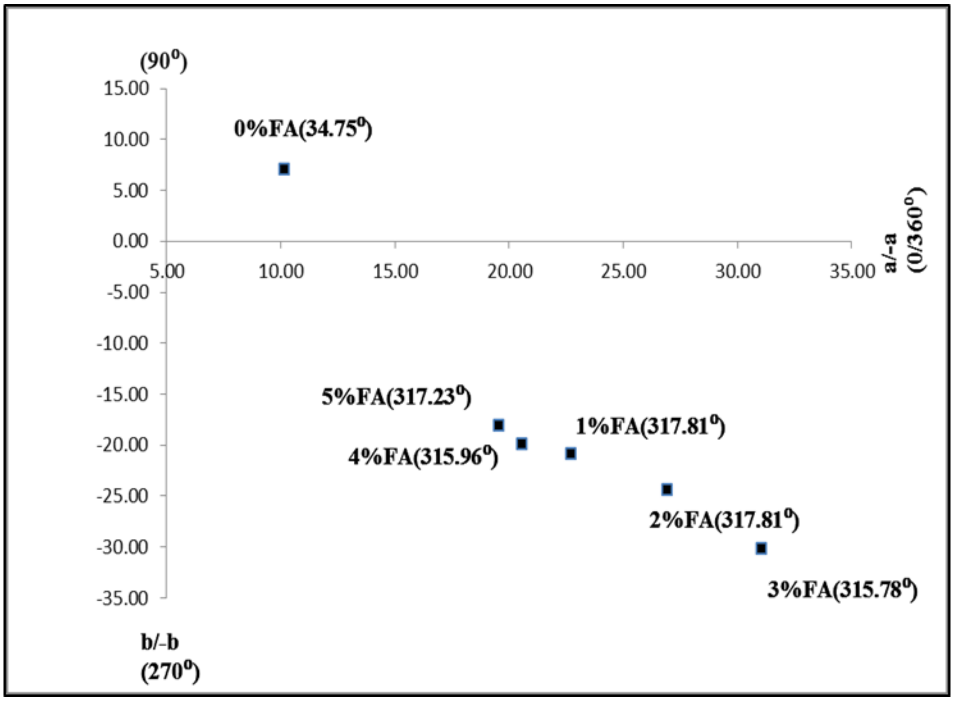


(a)



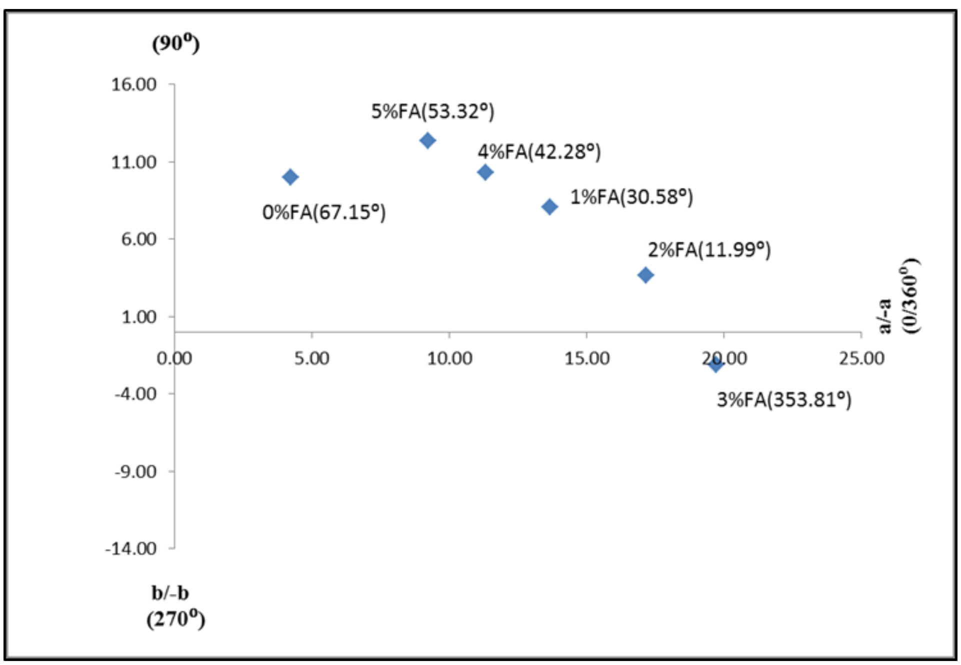
(b)

Figure 5.3: Relationship between percentage of FA and H° with a*b* co-ordinate for *M. malabathricum*-PVA blends during(a) 0 month (b) 1 month, (c) 2 month (d) 3 month of storage



(c)

'Figure 5.3, continued'



(d)

'Figure 5.3, continued'

Table 5.1: Influence of different percentage of FA on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin-PVA blend from *M.malabathricum*

FA(%)	**TIME (Month)**					
	0	**1**	**2**	**3**	**ΔE_1**	**ΔE_3**
0	s_0=0.2815	s_1=0.1991	s_2=0.1829	s_3=0.1380	ΔE_1=6.837	ΔE_3=29.047
1	s_0=0.3706	s_1=0.5835	s_2=0.6135	s_3=0.2448	ΔE_1=11.561	ΔE_3=25.062
2	s_0=0.4812	s_1=0.7703	s_2=0.8097	s_3=0.3053	ΔE_1=13.014	ΔE_3=23.149
3	s_0=0.6379	s_1=1.0771	s_2=1.1363	s_3=0.3890	ΔE_1=15.181	ΔE_3=21.995
4	s_0=0.3365	s_1=0.5183	s_2=0.5426	s_3=0.2242	ΔE_1=10.802	ΔE_3=27.020
5	s_0=0.3026	s_1=0.4627	s_2=0.4850	s_3=0.2164	ΔE_1=10.196	ΔE_3=28.111

The above result was further analysed interm of Total Colour difference (ΔE) and saturation(s).Table 5.1 displayed the results obtained from the analysis for crude anthocyanin–PVA blend with different addition FA. The saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s.According to the table, the different percentage of FA gave variation of colour and resulted in different saturation of colour for crude anthocyanin-PVA blend. Highest colour saturation recorded for the samples at 3% FA added (s_2=1.1363) in the 2nd month of the storage and gradually decreased during storage with the saturation recorded were s_3=0.3890 which the highest saturation recorded compared to the other samples. Consequently these results showed that samples containing 3% FA resulted in more vivid purple-blue colour compared to the other samples studied.However, as realized in the table the the lowest saturation value recorded for the non-enhance samples with the saturation was observed to decreased over the storage period from s_0=0.2815, to s_3=0.1380 .Therefore it directly noticed that, the visual colour of sample of non-enhance samples obviously bleached during the 3 month of the storage.

Furthermore, ΔE represents the colour change of three colour coordinate (C*, L* and h*) of sample before and after exposed to high UV irradiadiation for 3 month. Smallest (ΔE) was noticed for the samples with 0% FA, ΔE_1=6.837 at the beginning of the storage. After 3 month of the storage the ΔE_3=29.047 was increased for which showed the colour of samples varied compared to the first month with the saturation only s_3=0.1380. Based on the results obtained, it can clearly visible that the highest ΔE at the first month recorded for the sample with3% FA (ΔE_1=15.181) which showed that the crude anthocyanin-PVA blends was effectively enhanced**.**Nevertheless at the end of the storage ΔE of 3% FA samples was increased (21.995) but still most coloured than the others samples tested with the most saturated coloured (s_3=0.3890)

Table 5.2: Relationship between percentage of FA and L*C* a* and b* values for crude anthocyanin-PVA blend for *M.malabathricum*

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.1)	Minimum	Maximum
L*	0	0	63.813_{7}	.018765	63.781	63.846
		1	59.144_{10}	.014434	59.119	59.169
		2	54.910_{14}	.008950	54.895	54.926
		3	49.144_{20}	.007796	49.131	49.158
		4	61.154_{9}	.015301	61.128	61.181
		5	62.946_{8}	.011547	62.926	62.966
	1	0	67.014_{5}	.015588	66.987	67.041
		1	51.032_{17}	.013569	51.009	51.056
		2	45.623_{21}	.012991	45.601	45.646
		3	38.971_{23}	.008950	38.956	38.987
		4	53.343_{15}	.016166	53.315	53.371
		5	55.517_{12}	.017898	55.486	55.548
	2	0	67.535_{4}	.013856	67.511	67.559
		1	50.354_{19}	.012991	50.332	50.377
		2	44.915_{22}	.017033	44.886	44.945
		3	38.164_{24}	.010682	38.146	38.183
		4	52.755_{16}	.017610	52.725	52.786
		5	54.949_{13}	.015011	54.923	54.975
	3	0	78.704_{1}	.009815	78.687	78.721
		1	64.837_{6}	.015301	64.811	64.864
		2	57.461_{11}	.011837	57.441	57.482
		3	50.997_{18}	.011260	50.978	51.017
		4	68.247_{3}	.015301	68.221	68.274
		5	71.363_{2}	.012991	71.341	71.386

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.2, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.1)	Minimum	Maximum
c*	0	0	17.962_{17}	.011837	17.942	17.983
		1	21.918_{13}	.015878	21.891	21.946
		2	26.422_{11}	.017321	26.392	26.452
		3	31.349_{5}	.007219	31.337	31.362
		4	20.579_{14}	.017610	20.549	20.610
		5	19.047_{16}	.015301	19.021	19.074
	1	0	13.340_{22}	.011837	13.320	13.361
		1	29.775_{7}	.019919	29.741	29.810
		2	35.142_{4}	.011547	35.122	35.162
		3	41.977_{2}	.009528	41.961	41.994
		4	27.647_{9}	.014146	27.623	27.672
		5	25.689_{12}	.015011	25.663	25.715
	2	0	12.353_{23}	.010392	12.335	12.371
		1	30.893_{6}	.016166	30.865	30.921
		2	36.366_{3}	.014723	36.341	36.392
		3	43.368_{1}	.010105	43.351	43.386
		4	28.625_{8}	.014723	28.600	28.651
		5	26.648_{10}	.015878	26.621	26.676
	3	0	10.864_{24}	.017033	10.835	10.894
		1	15.869_{19}	.018187	15.838	15.901
		2	17.543_{18}	.016166	17.515	17.571
		3	19.835_{15}	.012414	19.814	19.857
		4	15.303_{21}	.016743	15.274	15.332
		5	15.441_{20}	.014723	15.416	15.467

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.2, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
h*	0	0	309.479_{18}	.011260	309.460	309.499
		1	311.900_{15}	.015301	311.874	311.927
		2	312.919_{14}	.015301	312.893	312.946
		3	309.900_{17}	.008373	309.886	309.915
		4	310.279_{16}	.015301	310.253	310.306
		5	311.740_{15}	.018475	311.708	311.772
	1	0	323.910_{2}	.013856	323.886	323.934
		1	317.460_{5}	.013856	317.436	317.484
		2	317.919_{3}	.019342	317.886	317.953
		3	315.819_{11}	.010682	315.801	315.838
		4	316.010_{9}	.013279	315.987	316.033
		5	317.320_{7}	.016743	317.291	317.349
	2	0	34.752_{22}	.016455	34.724	34.781
		1	317.359_{6}	.016455	317.331	317.388
		2	317.810_{4}	.014146	317.786	317.835
		3	315.780_{12}	.009815	315.763	315.797
		4	315.969_{10}	.020497	315.934	316.005
		5	317.230_{8}	.013856	317.206	317.254
	3	0	67.139_{19}	.015301	67.113	67.166
		1	30.580_{23}	.019053	30.547	30.613
		2	11.988_{24}	.013279	11.965	12.011
		3	353.810_{1}	.008083	353.796	353.824
		4	42.282_{21}	.010682	42.264	42.301
		5	53.317_{20}	.016166	53.289	53.345

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.2, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	0	11.421_{19}	.014146	11.397	11.446
		1	14.641_{15}	.017321	14.611	14.671
		2	17.994_{13}	.016743	17.965	18.023
		3	20.110_{8}	.012414	20.089	20.132
		4	13.306_{17}	.011837	13.286	13.327
		5	12.681_{18}	.011837	12.661	12.702
	1	0	10.780_{21}	.014723	10.755	10.806
		1	21.942_{6}	.015588	21.915	21.969
		2	26.085_{4}	.018475	26.053	26.117
		3	30.108_{2}	.009238	30.092	30.124
		4	19.894_{9}	.015301	19.868	19.921
		5	18.88812	.017033	18.859	18.918
	2	0	10.150_{22}	.010392	10.132	10.168
		1	22.728_{5}	.015878	22.701	22.756
		2	26.947_{3}	.015011	26.921	26.973
		3	31.080_{1}	.012414	31.059	31.102
		4	20.582_{7}	.016743	20.553	20.611
		5	19.563_{11}	.014723	19.538	19.589
	3	0	4.221_{24}	.018187	4.190	4.253
		1	13.663_{16}	.011547	13.643	13.683
		2	17.161_{14}	.011260	17.142	17.181
		3	19.721_{10}	.006642	19.710	19.733
		4	11.322_{20}	.014434	11.297	11.347
		5	9.224_{23}	.017610	9.194	9.255

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.2, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	0	-13.865_{16}	.017321	13.835	13.895
		1	-16.313_{13}	.012702	16.291	16.335
		2	-19.348_{9}	.014146	19.324	19.373
		3	-24.050_{4}	.008660	24.035	24.065
		4	-15.701_{14}	.013279	15.678	15.724
		5	-14.212_{15}	.018187	14.181	14.244
	1	0	-7.857_{21}	.014723	7.832	7.883
		1	-20.128_{7}	.015878	20.101	20.156
		2	-23.550_{5}	.016743	23.521	23.579
		3	-29.251_{2}	.011547	29.231	29.271
		4	-19.200_{10}	.018187	19.169	19.232
		5	-17.411_{12}	.017610	17.381	17.442
	2	0	-7.0422_{2}	.012414	7.021	7.064
		1	-20.923_{6}	.019400	20.890	20.957
		2	-24.423_{3}	.012702	24.401	24.445
		3	-30.245_{1}	.011837	30.225	30.266
		4	-19.896_{8}	.022229	19.858	19.935
		5	-18.096_{11}	.017321	18.066	18.126
	3	0	10.012_{19}	.015301	9.986	10.039
		1	8.073_{20}	.010105	8.056	8.091
		2	3.643_{23}	.014723	3.618	3.669
		3	2.136_{24}	.007219	2.124	2.149
		4	10.296_{18}	.016455	10.268	10.325
		5	12.384_{17}	.015588	12.357	12.411

(Note: Means with the different subscript numbers are significantly different at P<0.**05)**

5.2.2. Influence of different pH on Visual Colour Variation of purified anthocyanin-PVA blends

Figure 5.4 displays the influence of different pH (pH initial 5.8, pH 1,3, 7,9 and 11) on visual colour variation for crude fruit pulp of anthocyanin *M. malabathricum*-PVA blends in terms of L* (lightness).It can be noticed that from pH 1 the lightness percentage of anthocyanin *M. Malabathricum*-PVA blend (63.291±0.0112) decrease over increasing pH 11 (56.833±0.016) at zero time of storage. However, when approach pH 5.8, the lightness started to slightly increase (63.813±0.019) before decrease when pH reaching pH 7 (61.693±0.016) and continue follow the decreasing trend. In addition during the storage period, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (88.355±0.0184) after the 3 month of the storage. As expected, the slightly increased in L* value was observed for the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (76.515±0.013), (75.487±0.020) and (74.795±.0176) respectively.

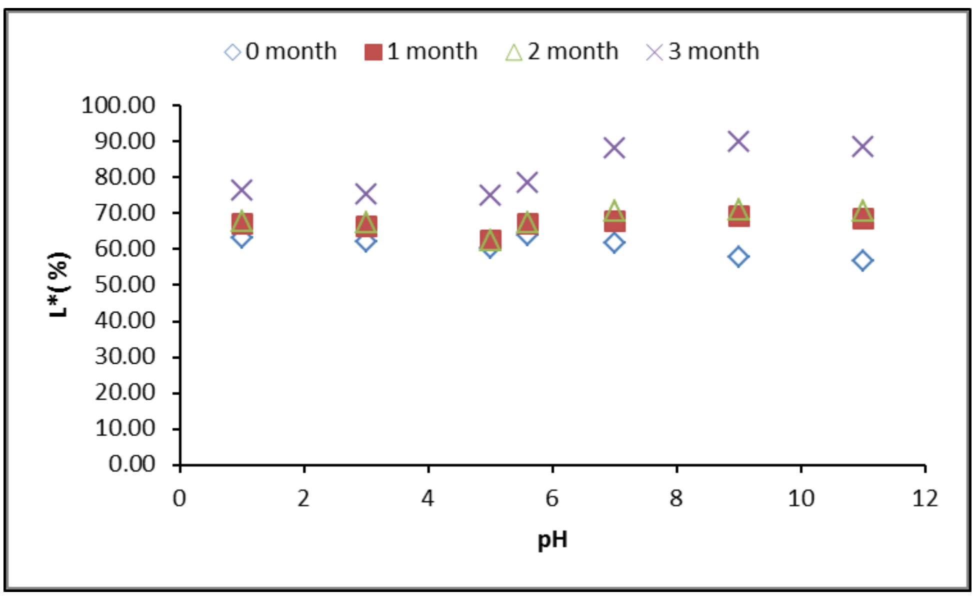


Figure 5.4: Relationship between pH variation and L* values (%) for *M. malabathricum*-PVA blends during 3 month of storage

Altered the pH for crude anthocyanin *M. malabathricum*-PVA blends also gave different chroma value. As seen in figure 5.5 the C* value for most acidic crude anthocyanin *M. malabathricum*-PVA blends,pH1 (57.888±.0138) recorded as the maximum chroma value compared to the other samples studied.Though, from the table It can be realised that, as starting pH 1 the chromaticity decrease (57.888±.0138) with increasing pH 11 (18.215±0.017) but when reaching pH 9 the chromaticity were slightly increase (36.869±0.0165).On the other hand, the chroma of the crude anthocyanin *M. malabathricum*-PVA blends also decreased during 3 month of storage which result in dull in colour. As seen in table, most coloured sample pH 1 also experience the colour fading by reducing in C* value over time and it obviously at the end of the storage, the C* value recorded was (48.705±0.015) which still resulted in the highest C* value (more brighter colour) compared to the other pH study. Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

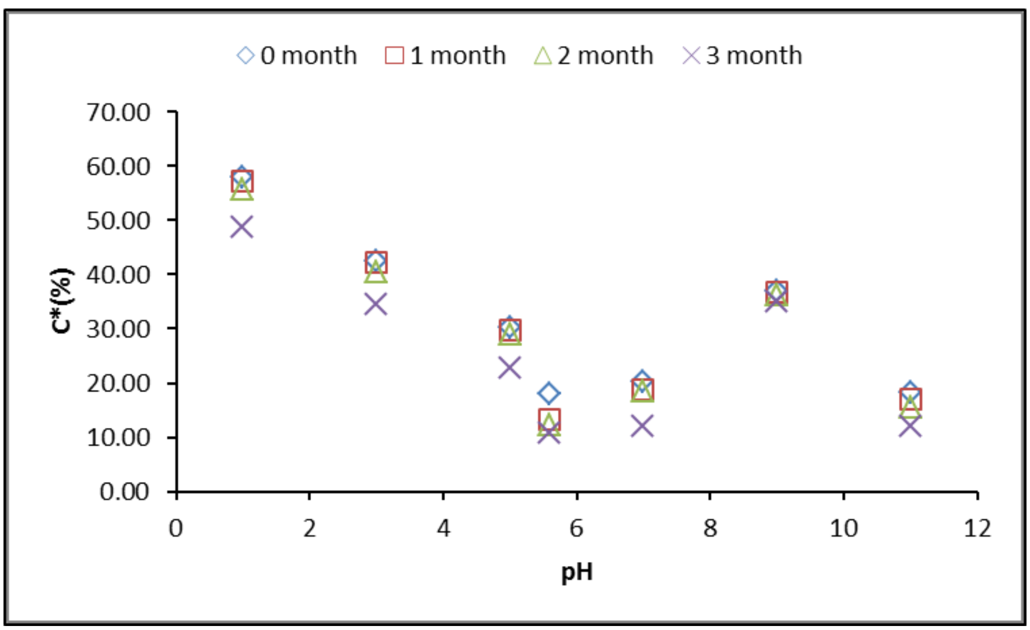
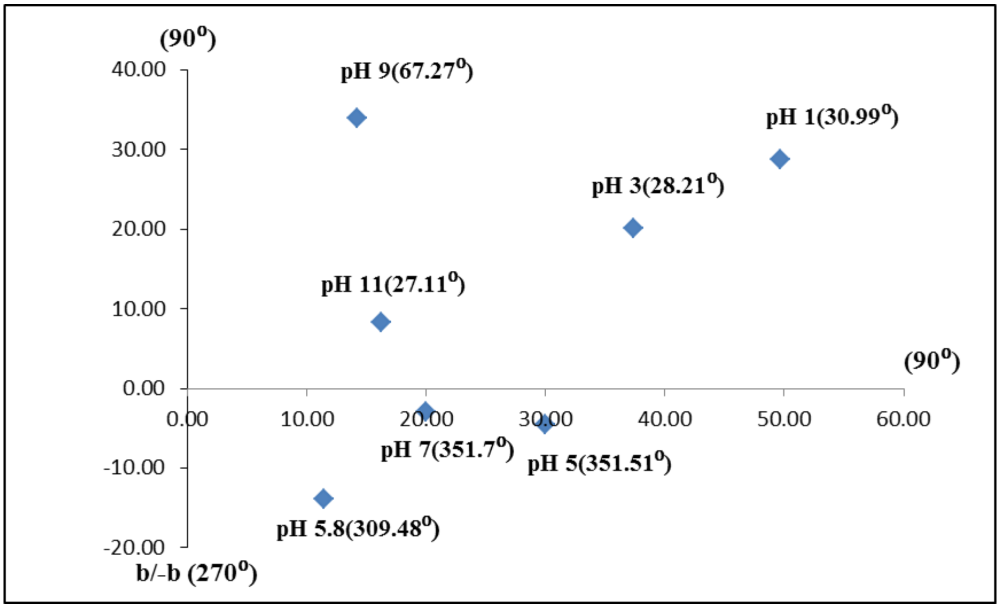


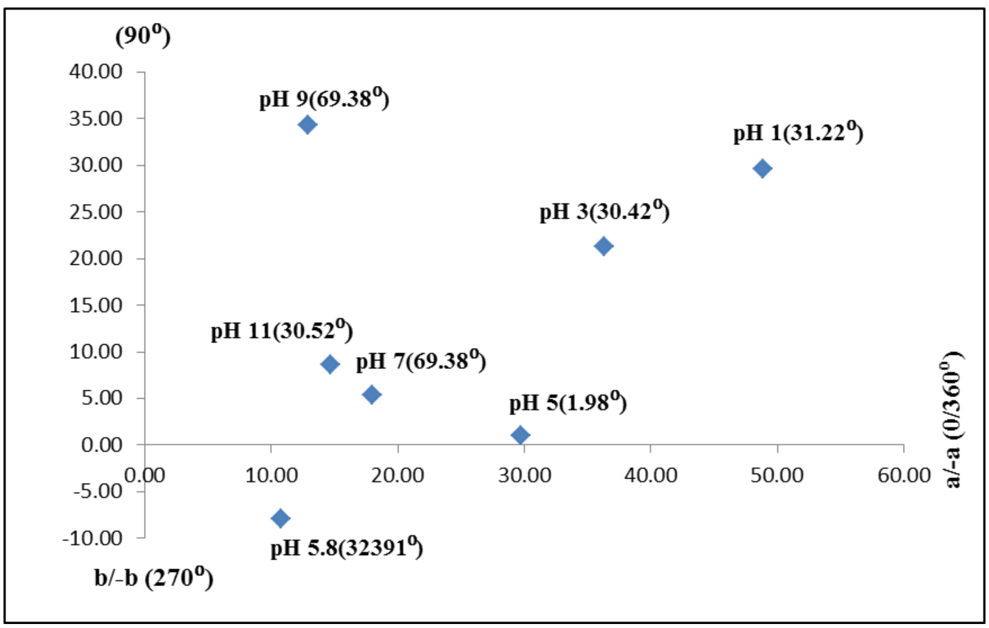
Figure 5.5: Relationship between pH variation and C* values (%) for *M. malabathricum*-PVA blends during 3 month of storage

Hue, which also vital affect caused by variation of pH. As in figure 5.6(a) to 5.6(d), Further augmentation of pH 1, H° (30.990±0.0170) to higher pH 5.8 caused an important counterclockwise shift of hue angle H° (309.479±.0112), meaning that the hues now moved back to yellower tonalities. Based on table 6.16, it shows that the pH 5.8 of *crude anthocyanin M. Malabathricum*-PVA blends present in positive a* values (11.421±.0147) and negative b* values (-13.865±.0173) with hue angle (h_{ab} 309.479±0.0113) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (49.624±.0135) and negative b* values moved to positive value (28.807±.0116) while hue angle moved clockwise to lower value (h_{ab} 49.625±.0136) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (14.242±.0173) and b* slightly increase to positive values (34.008±.01386) while hue angle moved counterclockwise (h_{ab} 67.276±.0136).

Furthermore, the pH variation also affects the visual colour stability of crude anthocyanin colourat during storage. Based on Table 5.3, it clearly noticed that the visual colour of samples with more alkaline pH easily faded during storage with the a* (4.471±.0217) moved to lower positive value and b* slightly moved to more yellower tonalities (11.110±.0092), while the hue angle moved counterclockwise (h_{ab} 68.078±0.0182) which showed the colour degradation of anthocyanin-PVA blends.Additionaly, the most coloured sample at pH 1 also experience calour degradation durig 3 month of the storage with the a* values started to move to the less positive(31.260±.0167) and b* increased to more positive value (37.349±.0141) with increasing the H° (50.072±.0156). Nonetheless it again can be noticed that, at the end of the storage, the pH 1 succesfully improved the colour stability of the crude colourant which resulted in the most coloured sample compared to the other pH study.

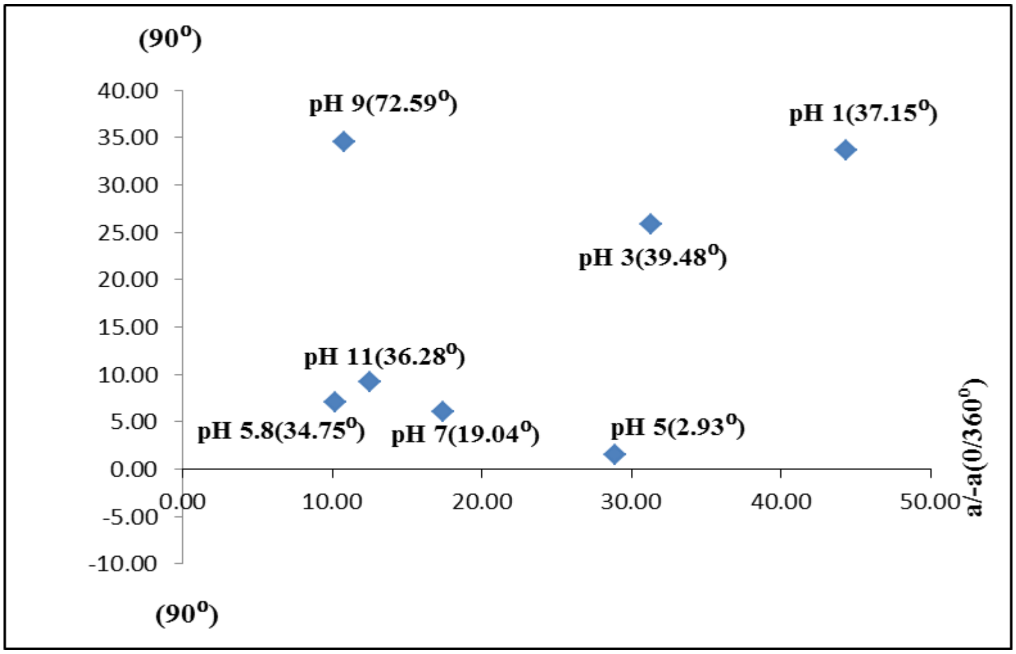


(a)



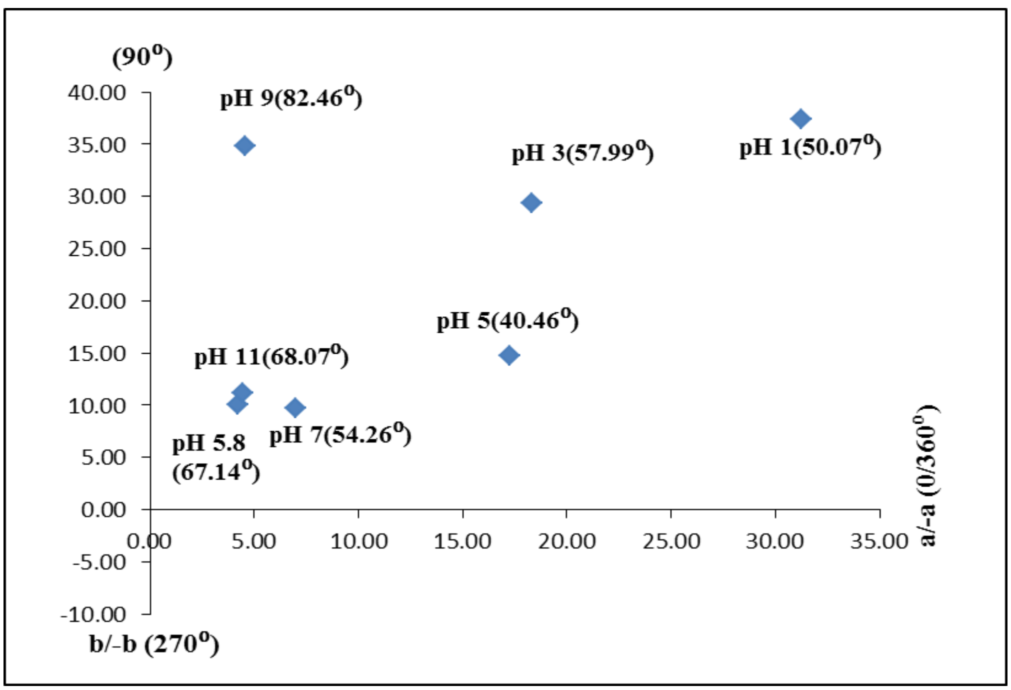
(b)

Figure 5.6: Relationship between pH variation and H◦ with a*b* co-ordinate for crude *M. malabathricum*-PVA blends during (a) 0 month, (b) 1 month, (c) 2 month and (d) 3 month of storage



(c)

'Figure 5.6, continued'



(d)

'Figure 5.6, continued'

Table 5.3: Relationship between pH variation and L*C* a* and b* values for crude anthocyanin-PVA blends *M. malabathricum*

CIELab value	Time (Month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
L*	0	1	63.291_{20}	.011260	63.272	63.311
		3	61.974_{23}	.012414	61.953	61.996
		5	60.281_{25}	.016166	60.253	60.309
		5.8	63.813_{19}	.018765	63.781	63.846
		7	61.693_{24}	.015186	61.667	61.720
		9	57.835_{26}	.014723	57.810	57.861
		11	56.833_{28}	.015878	56.806	56.861
	1	1	67.121_{16}	.011837	67.101	67.142
		3	66.498_{18}	.014146	66.474	66.523
		5	62.381_{22}	.010105	62.364	62.399
		5.8	67.014_{17}	.015588	66.987	67.041
		7	67.979_{13}	.018765	67.947	68.012
		9	69.135_{11}	.013569	69.112	69.159
		11	68.439_{12}	.015878	68.412	68.467
	2	1	67.923_{14}	.012991	67.901	67.946
		3	67.495_{15}	.015011	67.469	67.521
		5	62.593_{21}	.012702	62.571	62.615
		5.8	67.535_{15}	.013856	67.511	67.559
		7	70.784_{9}	.011547	70.764	70.804
		9	70.987_{8}	.019342	70.954	71.021
		11	70.598_{10}	.019053	70.565	70.631
	3	1	76.515_{5}	.012702	76.493	76.537
		3	75.487_{6}	.020497	75.452	75.523
		5	74.795_{7}	.017610	74.765	74.826
		5.8	78.704_{4}	.009815	78.687	78.721
		7	87.996_{3}	.018765	87.964	88.029
		9	90.025_{1}	.017033	89.996	90.055
		11	88.355_{2}	.018475	88.323	88.387

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.3, continued'

CIELab value	Time (Month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
C*	0	1	57.888_{1}	.013856	57.864	57.912
		3	42.45_{15}	.016166	42.423	42.479
		5	30.328_{13}	.015878	30.301	30.356
		5.8	17.962_{21}	.011837	17.942	17.983
		7	20.213_{17}	.009528	20.197	20.230
		9	36.869_{8}	.016455	36.841	36.898
		11	18.215_{20}	.017321	18.185	18.245
	1	1	57.18_{12}	.010392	57.163	57.199
		3	42.167_{6}	.010105	42.150	42.185
		5	29.714_{14}	.011547	29.694	29.734
		5.8	13.340_{24}	.011837	13.320	13.361
		7	18.786_{18}	.017610	18.756	18.817
		9	36.680_{9}	.016455	36.652	36.709
		11	17.022_{22}	.016455	16.994	17.051
	2	1	55.689_{3}	.015011	55.663	55.715
		3	40.553_{7}	.017033	40.524	40.583
		5	28.962_{15}	.015301	28.936	28.989
		5.8	12.353_{25}	.010392	12.335	12.371
		7	18.417_{19}	.011837	18.397	18.438
		9	36.207_{10}	.009528	36.191	36.224
		11	15.509_{23}	.012991	15.487	15.532
	3	1	48.705_{14}	.014723	48.680	48.731
		3	34.561_{12}	.013279	34.538	34.584
		5	22.697_{16}	.014146	22.673	22.722
		5.8	10.864_{27}	.017033	10.835	10.894
		7	11.995_{26}	.016455	11.967	12.024
		9	35.066_{11}	.018187	35.035	35.098
		11	11.975_{26}	.013856	11.951	11.999

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.3,continued'

CIELab value	Time (Month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
H*	0	1	30.990_{20}	.017033	30.961	31.020
		3	28.205_{23}	.009528	28.189	28.222
		5	351.510_{2}	.012124	351.489	351.531
		5.8	309.479_{4}	.011260	309.460	309.499
		7	351.700_{1}	.012702	351.678	351.722
		9	67.275_{9}	.013569	67.252	67.299
		11	27.112_{24}	.011260	27.093	27.132
	1	1	31.215_{19}	.014723	31.190	31.241
		3	30.420_{22}	.013569	30.397	30.444
		5	1.982_{28}	.009526	1.966	1.999
		5.8	323.910_{3}	.013856	323.886	323.934
		7	16.443_{26}	.012702	16.421	16.465
		9	69.376_{7}	.016455	69.348	69.405
		11	30.521_{21}	.014146	30.497	30.546
	2	1	37.151_{16}	.008950	37.136	37.167
		3	39.475_{15}	.013856	39.451	39.499
		5	2.928_{27}	.015878	2.901	2.956
		5.8	34.752_{18}	.016455	34.724	34.781
		7	19.040_{25}	.011547	19.020	19.060
		9	72.592_{6}	.021074	72.556	72.629
		11	36.283_{17}	.012702	36.261	36.305
	3	1	50.072_{13}	.015588	50.045	50.099
		3	57.985_{11}	.019342	57.952	58.019
		5	40.455_{14}	.018187	40.424	40.487
		5.8	67.139_{10}	.015301	67.113	67.166
		7	54.260_{5}	.011547	54.240	54.280
		9	82.457_{3}	.019342	82.424	82.491
		11	68.077_{8}	.018187	68.046	68.109

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.3, continued'

CIELab value	Time (Month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	1	49.624_{1}	.013569	49.601	49.648
		3	37.41_{14}	.016743	37.382	37.440
		5	29.998_{8}	.013856	29.974	30.022
		5.8	11.421_{21}	.014723	11.396	11.447
		7	20.001_{11}	.012414	19.980	20.023
		9	14.242_{16}	.017033	14.213	14.272
		11	16.213_{2}	.010105	16.196	16.231
	1	1	48.902_{5}	.012414	48.881	48.924
		3	36.36_{29}	.012702	36.340	36.384
		5	29.696_{23}	.014723	29.671	29.722
		5.8	10.780_{13}	.014723	10.755	10.806
		7	18.019_{19}	.015011	17.993	18.045
		9	12.919_{17}	.012991	12.897	12.942
		11	14.665_{3}	.015588	14.638	14.692
	2	1	44.38_{56}	.015878	44.358	44.413
		3	31.304_{10}	.012124	31.283	31.325
		5	28.926_{24}	.014434	28.901	28.951
		5.8	10.150_{14}	.010392	10.132	10.168
		7	17.411_{22}	.013856	17.387	17.435
		9	10.832_{20}	.012702	10.810	10.854
		11	12.502_{7}	.008950	12.487	12.518
	3	1	31.260_{12}	.016743	31.231	31.289
		3	18.321_{15}	.014723	18.296	18.347
		5	17.271_{28}	.014434	17.246	17.296
		5.8	4.221_{25}	.018187	4.190	4.253
		7	7.006_{25}	.011260	6.987	7.026
		9	4.602_{26}	.009238	4.586	4.618
		11	4.471_{27}	.021651	4.434	4.509

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.3, continued'

CIELab value	Time (Month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	1	28.807_{9}	.011547	28.787	28.827
		3	20.064_{12}	.012124	20.043	20.085
		5	-4.472_{25}	.011837	4.452	4.493
		5.8	-13.865_{14}	.017321	13.835	13.895
		7	-2.916_{26}	.015878	2.889	2.944
		9	34.008_{5}	.013856	33.984	34.032
		11	8.302_{20}	.012414	8.281	8.324
	1	1	29.635_{7}	.014434	29.610	29.660
		3	21.351_{11}	.016743	21.322	21.380
		5	1.028_{28}	.015878	1.001	1.056
		5.8	-7.857_{21}	.014723	7.832	7.883
		7	5.318_{24}	.018187	5.287	5.350
		9	34.330_{4}	.012991	34.308	34.353
		11	8.646_{19}	.013279	8.623	8.669
	2	1	33.633_{6}	.017898	33.602	33.664
		3	25.782_{10}	.015878	25.755	25.810
		5	1.480_{27}	.010682	1.462	1.499
		5.8	7.042_{22}	.012414	7.021	7.064
		7	6.009_{23}	.012991	5.987	6.032
		9	34.549_{3}	.013569	34.526	34.573
		11	9.178_{18}	.018765	9.146	9.211
	3	1	37.349_{1}	.014146	37.325	37.374
		3	29.305_{8}	.012702	29.283	29.327
		5	14.728_{13}	.017898	14.697	14.759
		5.8	10.012_{16}	.015301	9.986	10.039
		7	9.737_{17}	.013856	9.713	9.761
		9	34.762_{2}	.016455	34.734	34.791
		11	11.110_{15}	.009238	11.094	11.126

(Note: Means with the different subscript numbers are significantly different at P<0.05)

Table 5.4: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin-PVA blend from *M.malabathricum*

FA(%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=0.9146	s_1=0.8519	s_2=0.8199	s_3=0.6365	ΔE_1=3.985	ΔE_3=24.189
pH 3	s_0=0.6850	s_1=0.6341	s_2=0.6008	s_3=0.4578	ΔE_1=4.818	ΔE_3=25.147
pH 5	s_0=0.5031	s_1=0.4763	s_2=0.4627	s_3=0.3035	ΔE_1=5.896	ΔE_3=27.227
pH 5.8	s_0=0.2815	s_1=0.1991	s_2=0.1829	s_3=0.1380	ΔE_1=6.837	ΔE_3=29.047
pH 7	s_0=0.3276	s_1=0.2764	s_2=0.2602	s_3=0.1363	ΔE_1=10.549	ΔE_3=31.951
pH 9	s_0=0.6375	s_1=0.5305	s_2=0.5100	s_3=0.3895	ΔE_1=11.383	ΔE_3=32.877
pH 11	s_0=0.3205	s_1=0.2487	s_2=0.2197	s_3=0.1355	ΔE_1=11.714	ΔE_3=33.755

The above result was further analysed interm of Total Colour difference (ΔE) and saturation(s).Table 5.4 displayed the results obtained from the analysis for crude anthocyanin–PVA blend with variation of pH. The saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. Furthermore, ΔE represents the colour change of three colour coordinate (C*, L* and h*) of sample before and after exposed to high UV irradiadiation for 3 month According to the table, the different pH variation gave different of colour and resulted in different saturation and ΔE of colour for crude anthocyanin-PVA blend. Smallest (ΔE) was noticed for the samples with pH 1 ΔE_1=3.985bat 1st month of the storage. After 3 month of the storage the ΔE_3=24.189

was increased for pH 1 which showed the colour change compared to the first month. Conferring to the table, it can clearly noticed that the highest ΔE at the first month recorded for the sample at pH 11 (11.714) and at the end of the storage pH 11 gave the highest total color change compared to the other samples tested (ΔE_3=33.755) which showed the huge colour different.

Highest colour saturation recorded for the samples at pH 1 (s_0=0.9146) and gradually decreased during storage with the saturation recorded were s_1=0.8519, s_2=0.8199,s_3=0.6365 correspondingly which showed that pH 1 resulted in more saturated red colour compared to the other acidic pH. Though the decreasing of the saturation over 3 month of storage period showed that colour of pH 1 samples also degraded.However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.3205 to s_3=0.1355at the end of the storage. Therefore it directly perceived that, the colour of sample at pH 11 obviously colourless during the 3 month of the storage.

5.2.3. Influence of different pH on Visual Colour Variation of crude anthocyanin-PVA blends containing 3% FA

Influence of different pH (pH initial 5.7, pH 1, 3, 7, 9 and 11) on visual colour variation for crude fruit pulp of anthocyanin *M. malabathricum*-PVA containing 3% FA displays in Table 5.5.Parameter in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) was studied.It can be seen that the lightness percentage decreased with increased in pH from pH 1 (52.852±0.006) to pH 11 (41.802±0.008) at zero time of storage. On the other hand, when approach pH 5.7 the lightness started to slightly increase (49.144±0.008) before continued decreased when pH approach pH 7 (46.872±0.008). According to figure 5.7, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (66.303±0.012) after the 3 month of the storage.Based on the results gained, the colour stability of the crude anthocyanin colourant degraded during the storage period under UVB- irradiation by increasing the the L* value which was resulted in lighter colour and the end of the storage pH 3 presents the most colour sample with L* value (50.943±0.008)

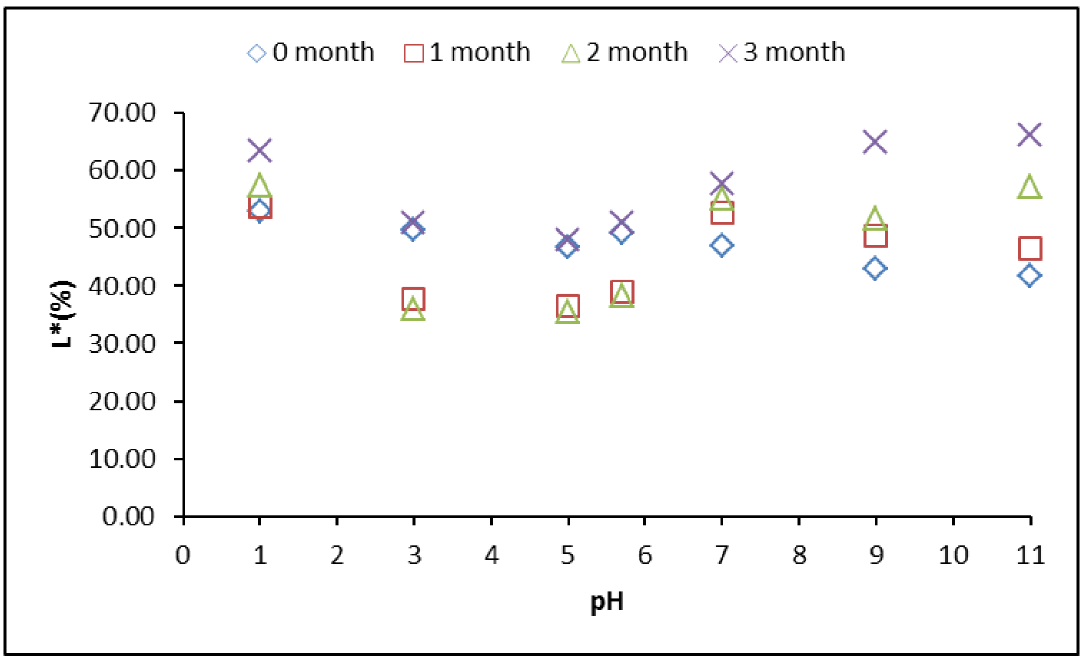


Figure 5.7: Relationship between pH variation and L* values (%) for *M. malabathricum*-PVA blends containing 3% FA during 3 month of storage

Besides, altered the pH for crude anthocyanin-PVA blends containing 3% FA also affected the colour chromaticity. As seen in figure 5.8 the C* value for most acidic crude anthocyanin colourant pH1 (55.306±0.011) recorded as the highest chroma compared to the other sample at the beginning the storage. However, from the table It can be seen as starting pH 1 the chromaticity decrease (55.306±0.011) with increasing pH 11 (19.861±0.012) which showed the huge different in C* nevertheless when reaching pH 9 the chromaticity were slightly increase (19.861±0.009).On the hand, the chroma of the purified anthocyanin colourant also decreased during 3 month of storage which result in dull in colour. As observed in table, most coloured sample resulted for the sample at pH 3, the C* value recorded was (41.658±0.007) which gave in the highest C* value (brighter colour) at the end of storage compared to the other pH study. The most coloured sample at the beginning of the storage did not retained the colour stability which the colour of the samples strongly faded at the end of the storage (33.171±0.010).Similar trend was also observed for the origin sample without adjusted pH which the C* value are also decreased over the storage period, and showed better results compared to alkaline crude colourant pH 11 (14.326±0.007). Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

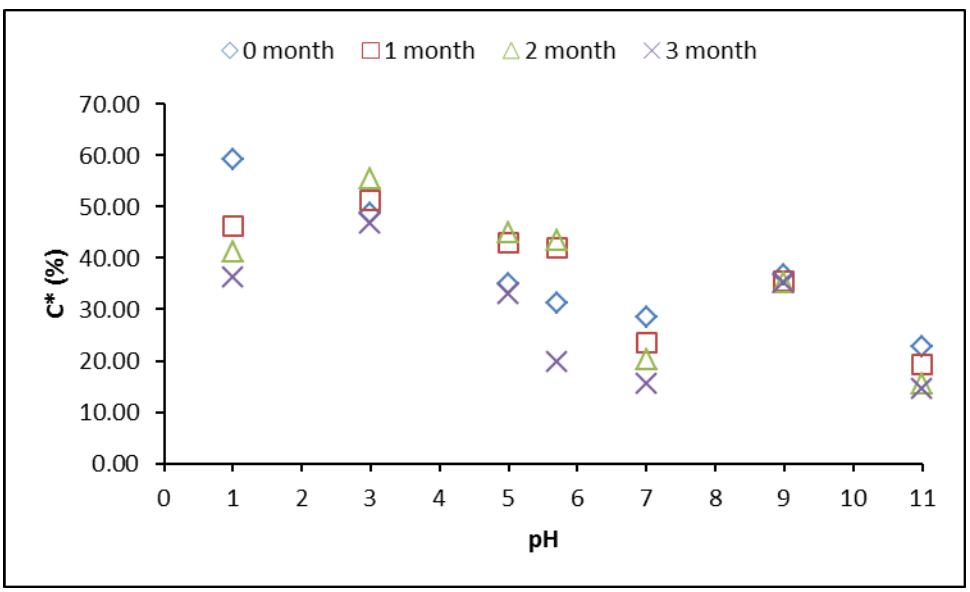
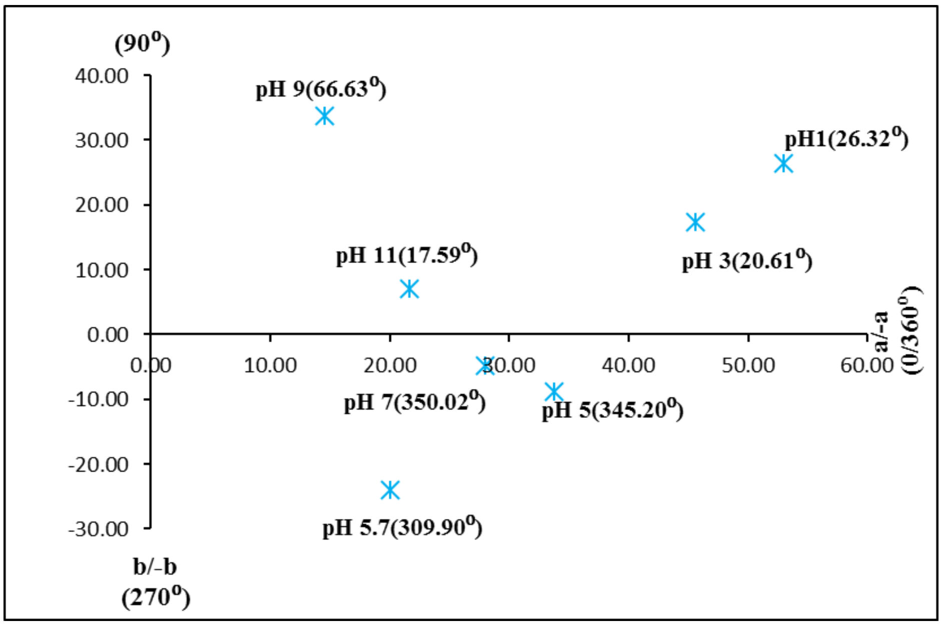


Figure 5.8: Relationship between pH variation and C* values (%) for *M. malabathricum*-PVA blends containing 3% FA during 3 month of storage

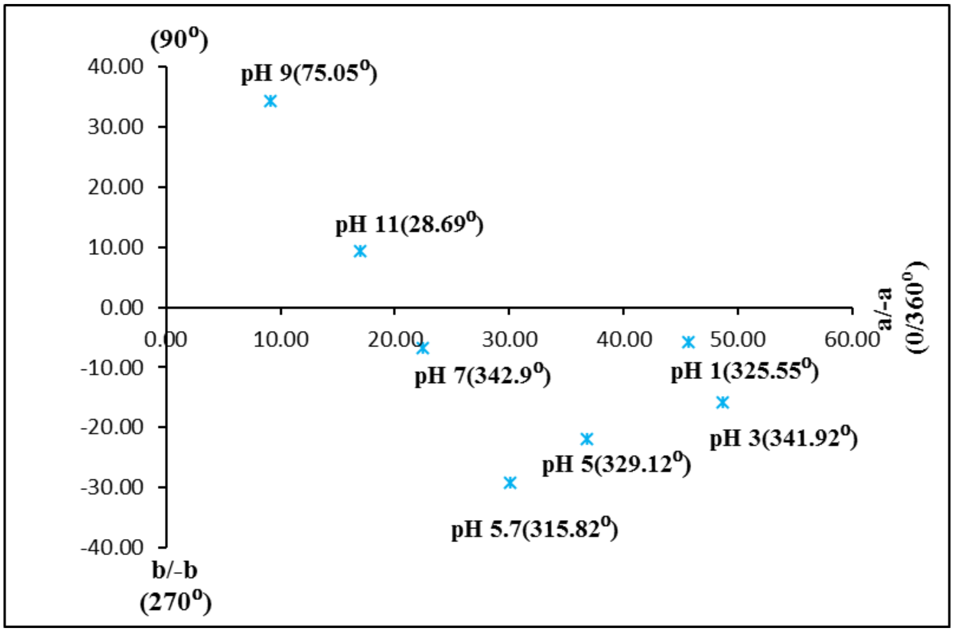
Besides, as listed in table 5.5, It shows that the pH 5.5 of *Melastoma malabathricum* present in positive a* values (19.928±0.009) and negative b* values (-22.871±0.010) with hue angle H^o (311.060±0.011) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (49.849±0.007) and negative b* values moved to positive value (23.956±0.006) while hue angle moved clockwise to lower value H^o (25.667 ±0.012) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (14.344±0.008) and b* slightly increase to positive values (28.140±0.008) while hue angle moved counterclockwise H^o (17.786±0.007).

pH variation also affects the visual colour stability of crude anthocyanin colourat during storage as plot in figure 5.9(a) to figure 5.9(d) which the highest colour enhancement was observed from the table for the sample with pH 3 with the 1st month, figure 5.7(a) increased in hue angle to (341.920±0.005) with a* positively increased (48.670±0.012) with positive b* value moved to negative b* value (-9.878±0.008) and slightly decreased h° in 2^{nd} month, figure 5.7(c) (340.310±0.010) with a* positively increased (49.871±0.008) and negative b* value (-16.984±0.006). pH 3 retained the colours stability after three month of the storage. Based on the table 5.5 also, it clearly noticed that the visual colour of samples with more alkaline pH 11 easily faded during storage with the a* (4.601±0.012) moved to lower positive value and b* slightly moved to more yellower tonalities (13.568±0.012), while the hue angle moved counterclockwise H^0 (65.059±0.010) which showed the colour degradation of anthocyanin colourant containing 3% FA. Moreover, In contrast for crude colourant containing 3% FA, the most coloured sample at pH 1 in the beginning of the storage obviously experience calour degradation during 3 month of the storage compared to pH 5 and pH 5.4 with the a* values started to move to the less positive (21.851±0.005) and b* decreased to more positive value (23.956±0.006) with increasing the H^0 (46.270±0.008). Nonetheless it again can be noticed that, at the end of the storage, the pH 3 succesfully improved the colour stability of the crude colourant containing 3FA which resulted in the most brighter colour compared to the other pH study.



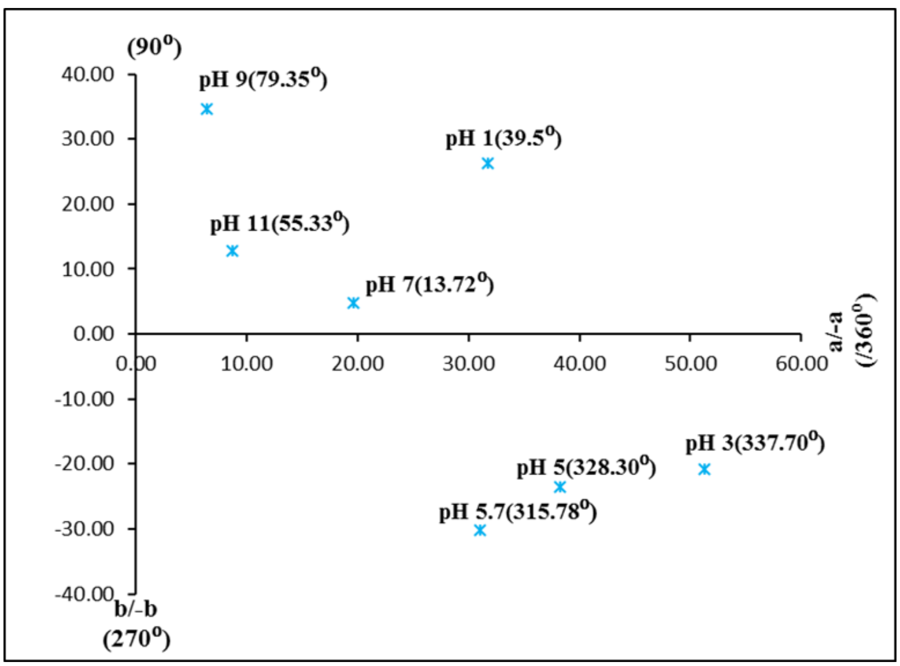
(a)

Figure 5.9:Relationship between pH variation and H◦ with a*b* co-ordinate for crude *M. malabathricum*-PVA blends containing 3%FA during (a) 0 month, (b) 1 month, (c) 2 month and (d) 3 month of storage



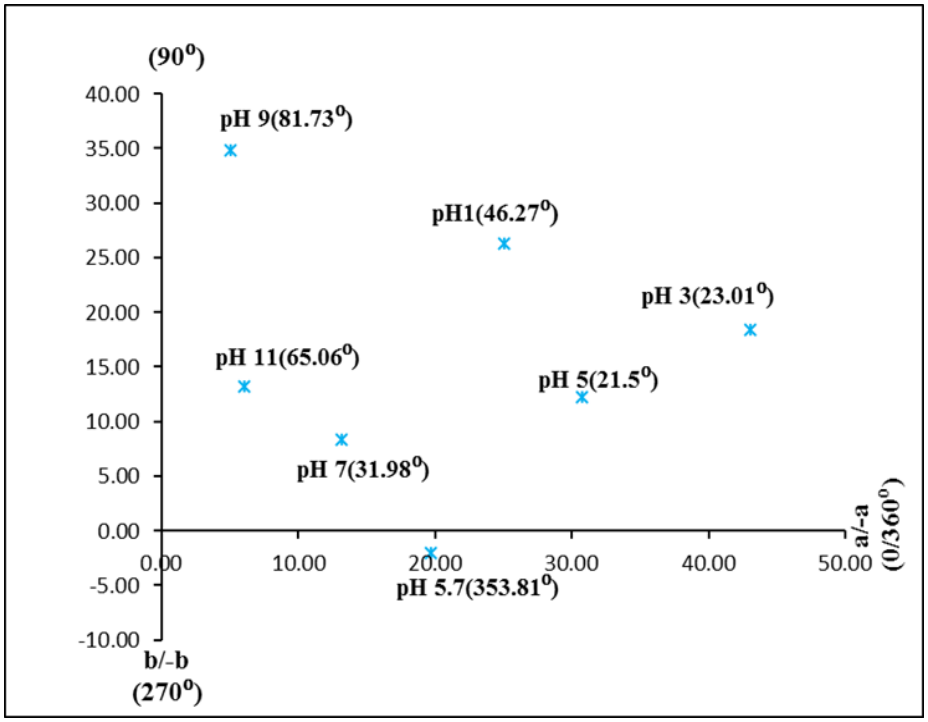
(b)

'Figure 5.9, continued'



(c)

'Figure 5.9, continued'



(d)

'Figure 5, continued'

Table 5.5: Relationship between pH variation and L*C* a* and b* values for crude anthocyanin-PVA blends *M. malabathricum* containing 3% FA

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
L*	0	1	52.852_{10}	0.006	52.842	52.862
		3	49.766_{13}	0.007	49.753	49.778
		5	46.673_{20}	0.007	46.661	46.686
		5.7	49.144_{14}	0.008	49.131	49.158
		7	46.872_{21}	0.008	46.858	46.886
		9	42.997_{26}	0.008	42.982	43.011
		11	41.802_{23}	0.008	41.789	41.816
	1	1	53.675_{8}	0.008	53.661	53.689
		3	37.677_{27}	0.006	37.667	37.688
		5	36.575_{24}	0.007	36.563	36.586
		5.7	38.972_{17}	0.009	38.956	38.987
		7	52.750_{16}	0.005	52.741	52.760
		9	48.797_{22}	0.011	48.778	48.816
		11	46.403_{18}	0.010	46.386	46.421
	2	1	57.480_{6}	0.006	57.469	57.491
		3	35.954_{28}	0.007	35.942	35.965
		5	35.373_{25}	0.006	35.363	35.384
		5.7	38.165_{18}	0.011	38.146	38.183
		7	55.337_{9}	0.008	55.323	55.352
		9	51.780_{13}	0.010	51.762	51.797
		11	57.216_{7}	0.011	57.196	57.235
	3	1	63.365_{3}	0.005	63.357	63.373
		3	50.943_{11}	0.008	50.929	50.956
		5	47.856_{15}	0.007	47.843	47.868
		5.7	50.997_{5}	0.011	50.978	51.017
		7	57.745_{4}	0.008	57.731	57.759
		9	64.849_{2}	0.013	64.827	64.872
		11	66.303_{1}	0.012	66.281	66.324

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.5, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
C*	0	1	59.138_{1}	0.008	59.124	59.151
		3	48.723_{6}	0.004	48.716	48.731
		5	34.945_{16}	0.008	34.931	34.958
		5.7	31.350_{10}	0.007	31.337	31.362
		7	28.491_{18}	0.011	28.471	28.510
		9	36.691_{13}	0.010	36.674	36.708
		11	22.728_{21}	0.011	22.710	22.747
	1	1	46.111_{10}	0.006	46.101	46.121
		3	51.240_{3}	0.008	51.227	51.254
		5	42.860_{9}	0.007	42.849	42.872
		5.7	41.977_{5}	0.010	41.961	41.994
		7	23.559_{19}	0.007	23.547	23.571
		9	35.502_{13}	0.011	35.482	35.521
		11	19.339_{22}	0.012	19.318	19.359
	2	1	41.243_{11}	0.007	41.231	41.256
		3	55.378_{2}	0.008	55.365	55.391
		5	44.985_{3}	0.010	44.968	45.002
		5.7	43.368_{4}	0.010	43.351	43.386
		7	20.196_{20}	0.010	20.179	20.212
		9	35.224_{14}	0.009	35.208	35.239
		11	15.429_{24}	0.009	15.413	15.445
	3	1	36.337_{12}	0.009	36.321	36.352
		3	46.843_{7}	0.005	46.834	46.853
		5	33.089_{17}	0.006	33.078	33.099
		5.7	19.836_{15}	0.012	19.814	19.857
		7	15.607_{23}	0.010	15.589	15.624
		9	35.197_{14}	0.008	35.184	35.211
		11	14.525_{25}	0.007	14.513	14.537

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.5, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
H*	0	1	26.315_{24}	0.006	26.304	26.326
		3	20.612_{27}	0.012	20.591	20.632
		5	345.200_{5}	0.007	345.189	345.212
		5.7	309.900_{4}	0.008	309.886	309.915
		7	350.020_{1}	0.011	350.001	350.038
		9	66.625_{17}	0.007	66.613	66.637
		11	17.590_{28}	0.010	17.572	17.607
	1	1	352.550_{2}	0.008	352.537	352.563
		3	341.920_{6}	0.005	341.911	341.929
		5	329.120_{10}	0.005	329.111	329.130
		5.7	315.820_{8}	0.011	315.801	315.838
		7	342.900_{3}	0.010	342.883	342.918
		9	75.054_{16}	0.012	75.034	75.074
		11	28.696_{26}	0.008	28.683	28.709
	2	1	39.499_{20}	0.006	39.488	39.509
		3	337.760_{7}	0.007	337.748	337.772
		5	328.380_{11}	0.008	328.367	328.394
		5.7	315.780_{9}	0.010	315.763	315.797
		7	13.719_{21}	0.008	13.705	13.732
		9	79.359_{14}	0.010	79.342	79.375
		11	55.330_{19}	0.011	55.312	55.349
	3	1	46.270_{18}	0.008	46.257	46.284
		3	23.015_{25}	0.005	23.006	23.025
		5	21.503_{22}	0.007	21.491	21.514
		5.7	353.810_{23}	0.008	353.796	353.824
		7	31.979_{15}	0.009	31.963	31.995
		9	81.726_{12}	0.009	81.710	81.742
		11	65.059_{13}	0.010	65.042	65.076

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.5, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	1	53.010_{1}	0.009	52.994	53.026
		3	45.604_{4}	0.006	45.593	45.614
		5	33.787_{12}	0.006	33.777	33.797
		5.7	20.11_{18}	0.012	20.089	20.132
		7	28.061_{13}	0.010	28.044	28.077
		9	14.557_{29}	0.010	14.539	14.574
		11	21.666_{17}	0.008	21.652	21.679
	1	1	45.723_{7}	0.007	45.711	45.734
		3	48.712_{3}	0.009	48.697	48.727
		5	36.788_{10}	0.006	36.778	36.799
		5.7	30.108_{7}	0.009	30.092	30.124
		7	22.518_{23}	0.009	22.502	22.533
		9	9.156_{16}	0.008	9.143	9.169
		11	16.964_{19}	0.010	16.946	16.981
	2	1	31.825_{14}	0.005	31.816	31.834
		3	51.259_{2}	0.006	51.248	51.269
		5	38.309_{9}	0.009	38.293	38.325
		5.7	31.081_{5}	0.012	31.059	31.102
		7	19.620_{20}	0.008	19.606	19.635
		9	6.504_{24}	0.008	6.489	6.518
		11	8.777_{22}	0.009	8.761	8.792
	3	1	25.119_{18}	0.008	25.105	25.133
		3	43.115_{6}	0.008	43.101	43.130
		5	30.786_{15}	0.007	30.774	30.798
		5.7	19.721_{11}	0.007	19.710	19.733
		7	13.239_{25}	0.008	13.225	13.253
		9	5.065_{26}	0.013	5.043	5.088
		11	6.125_{26}	0.005	6.116	6.134

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.5, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	1	26.217_{18}	0.008	26.203	26.232
		3	17.153_{1}	0.010	17.135	17.171
		5	-8.922_{17}	0.008	8.909	8.936
		5.7	-24.050_{16}	0.009	24.035	24.065
		7	-4.937_{12}	0.009	4.921	4.953
		9	33.680_{15}	0.010	33.662	33.697
		11	6.869_{5}	0.010	6.852	6.885
	1	1	-5.9732_{1}	0.006	5.962	5.984
		3	-15.896_{2}	0.007	15.884	15.908
		5	-21.993_{19}	0.010	21.976	22.011
		5.7	-29.25_{18}	0.012	29.231	29.271
		7	-6.926_{9}	0.006	6.916	6.937
		9	34.302_{13}	0.012	34.280	34.323
		11	9.286_{6}	0.011	9.267	9.306
	2	1	26.234_{24}	0.005	26.224	26.243
		3	-20.958_{3}	0.011	20.939	20.976
		5	-23.582_{26}	0.006	23.571	23.592
		5.7	-30.246_{10}	0.012	30.225	30.266
		7	4.7901_{1}	0.007	4.779	4.802
		9	34.619_{14}	0.012	34.599	34.639
		11	12.690_{25}	0.007	12.679	12.702
	3	1	26.258_{26}	0.008	26.244	26.273
		3	18.315_{4}	0.009	18.299	18.331
		5	12.129_{27}	0.004	12.121	12.136
		5.7	2.137_{22}	0.007	2.124	2.149
		7	8.266_{23}	0.011	8.247	8.284
		9	34.831_{20}	0.009	34.816	34.846
		11	13.171_{7}	0.010	13.153	13.189

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

Table 5.6: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of crude anthocyanin-PVA blends from *M.malabathricum* containing 3% FA

FA (%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=1.1189	s_1=0.8591	s_2=0.7175	s_3=0.5734	ΔE_1=33.015	ΔE_3=29.807
pH 3	s_0=0.9790	s_1=1.3600	s_2=1.5402	s_3=0.9195	ΔE_1=35.328	ΔE_3=2.988
pH 5	s_0=0.7487	s_1=1.1718	s_2=1.2717	s_3=0.6914	ΔE_1=16.788	ΔE_3=21.297
pH 5.7	s_0=0.6379	s_1=1.0771	s_2=1.1363	s_3=0.3890	ΔE_1=15.181	ΔE_3=21.995
pH 7	s_0=0.6078	s_1=0.4466	s_2=0.3650	s_3=0.2703	ΔE_1=8.321	ΔE_3=22.633
pH 9	s_0=0.8533	s_1=0.7275	s_2=0.6803	s_3=0.5427	ΔE_1=7.950	ΔE_3=23.852
pH 11	s_0=0.5437	s_1=0.4168	s_2=0.2697	s_3=0.2191	ΔE_1=7.009	ΔE_3=29.691

Table 5.6 displays the Total Colour difference (ΔE) of crude anthocyanin-PVA blend containing 3% FA with different pH (pH initial 5.9, pH 1, 3, 7, 9 and 11). Since the a* and b* parameters represent the redness and the yellowness on the chromaticity dimension, while the c* and h$^{\circ}$ parameter represent the Hunter a* and b* parameters and the ΔE represents the colour change of three colour coordinate (C*, L* and h) of sample before and after exposed to high UV irradiadiation for 3 month. In addition Saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, and effectively reduces the CIELab coordinates from three to single value, s. Smallest (ΔE) was noticed for the samples with pH 11 ΔE_1=7.009 at the beginning of the storage. After 3 month of the storage the ΔE_3=29.691 was increased for pH 11 which

showed the colour varied compared to the first month with the saturation s_3=0.2191. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample at pH 3 (ΔE_1=35.328) with the higher saturation s_0=0.9790 which showed that the crude anthocyanin-PVA blend was successfully enhanced. However at the end of the storage pH 3 samples results in small colour changes (ΔE_3=3.639) but still most coloured than the others samples tested with the saturation recorded s_3=0.9195

According to the table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.4, pH 1, 3, 7, 9 and 11) for crude anthocyanin-PVA containing 3% FA. From the table above the huge colour variation with the adjusting pH was observed. Highest colour saturation recorded for the samples at pH 3 (s_2=1.5402) in the 2nd month of the storage and gradually decreased during storage with the saturation recorded were s_3=0.9195 which the highest saturation recorded compared to the other samples. Hence these results showed that pH 3 resulted in more saturated purple-blue colour compared to the other pH studied. However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.5437to s_3=0.5427 at the end of the storage. Therefore it directly noticed that, the colour of sample at pH 11 obviously lost the colour during the 3 month of the storage.

5.3. Colour Analysis of purified anthocyanin-PVA blends from Fruit Pulp of *M. malabathricum*

5.3.1. Influence of different percentage of FA added on Visual Colour Variation (purified anthocyanin-PVA blend)

Influence of different percentage of FA addition for crude fruit pulp of anthocyanin *M. Malabathricum*-PVA blends on the values of the colour parameters (colorimetric indexes and CIELAB variables) in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) present in Tables 5.7. Similar tendency can be obsereved as crude anthocyanin colourant that at zero time strorage, the non-enhance purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends (0% FA) resulted in the lightest samples with highest L* (69.009±0.012). However addition of FA (1%, 2%, 3%, 4% and 5% FA) significantly decreased the L* value and samples with the addition of 3% FA gaved the lowest L* value (54.457±0.012) followed by 2% FA added (60.090±0.017). Figure 5.10 presents the plot of L* against percentage of FA added. It can be seen that the lightness of the non-enhance purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends blends slightly increased upon storage under 100% lux intensity (17.55 lux), with the end of storage (3 month) the L* value recorded was (91.130±0.015) for non-enhance samples. These results inferred that the colour of non-enhance samples lighter after 3 month of storage compared to the samples containing FA. Furthermore the colour stability of purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends improved with the addition of 3% FA with the lightness, L* of the sample decreased in 1 and 2 month of storage, however increased in L* value was observed during the last period of samples storage (58.874±0.008) meaning that colour of the 3% FA added slighly faded during 3 month of storage.

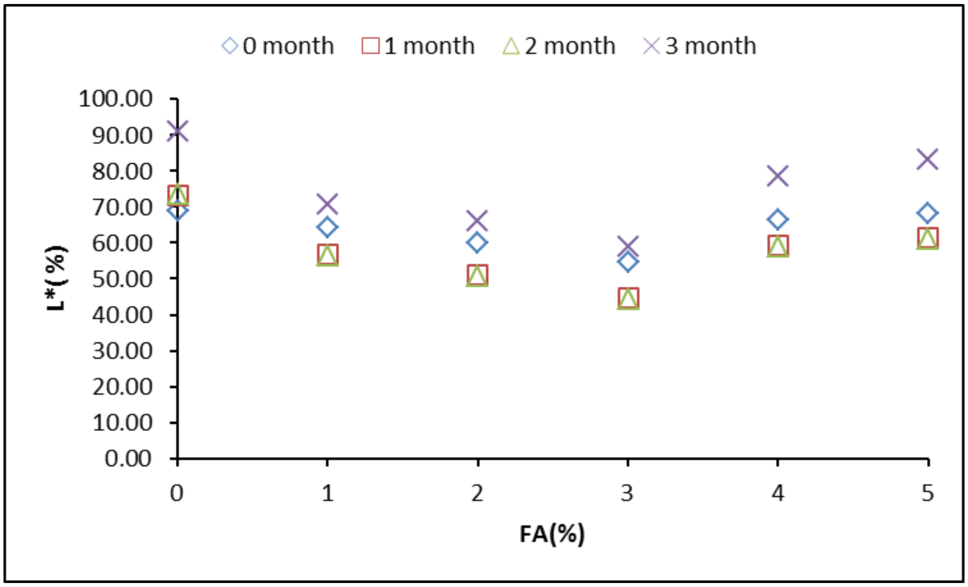


Figure 5.10: Relationship between percentage of FA and L* values (%) for purified *M. malabathricum*-PVA blends FA during 3 month of storage

As seen in figure 5.11 different addition of FA percentage also affects the colour chromaticity, C* values during 3 month of storage. At zero time storage the C* of non-enhance samples resulted in dull colour with the lowest C* value (13.807±0.012). On the other hand, addition of FA positively augmented the C* value with resulted in brighter colour. According to the table below, purified anthocayanin-PVA with the addition of 3% FA gave the brighter colour with highest C* value (30.334±0.012) compared to the other samples studied. Also, as seen in table further increased in FA % addition up to 4 and 5% FA resulted in decreased of C* value (15.925±0.017) and (15.342±0.017) respectively .The chroma results for purified anthocyanin-PVA blends was observed to decreased over the storage period for non-enhance samples. Additionally the C* for purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends with the addition of FA exhibit slightly increased upon storage up to 2 month before expericed decreased in C* at the end of the storage. This trend was obviously for 3% FA added crude colourant which the C* value increased over 2 month of storage (38.734±0.006), though, extended the storage period up to 3 month resulted in significantly decreased in C* value (19.836±0.012). The results gained for this investigation showed that 3% FA significantly enhance the colour of purified anthocyanin PVA blends by increased the C* value at the beginning of storage. Nevertheless, as non-enhance sample at the end of storage, the colour of 3% FA added samples also faded with resulted in decreased the C* value.On the other hand, at the end of storage, 3% FA added still resulted in the highest C* (16.442±0.009) value which means more coloured compared to the others.

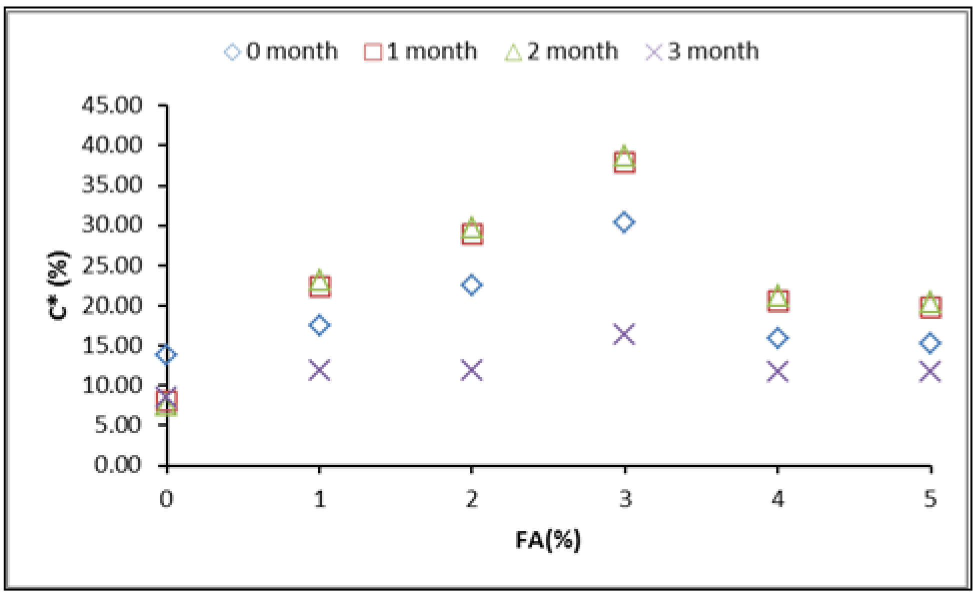
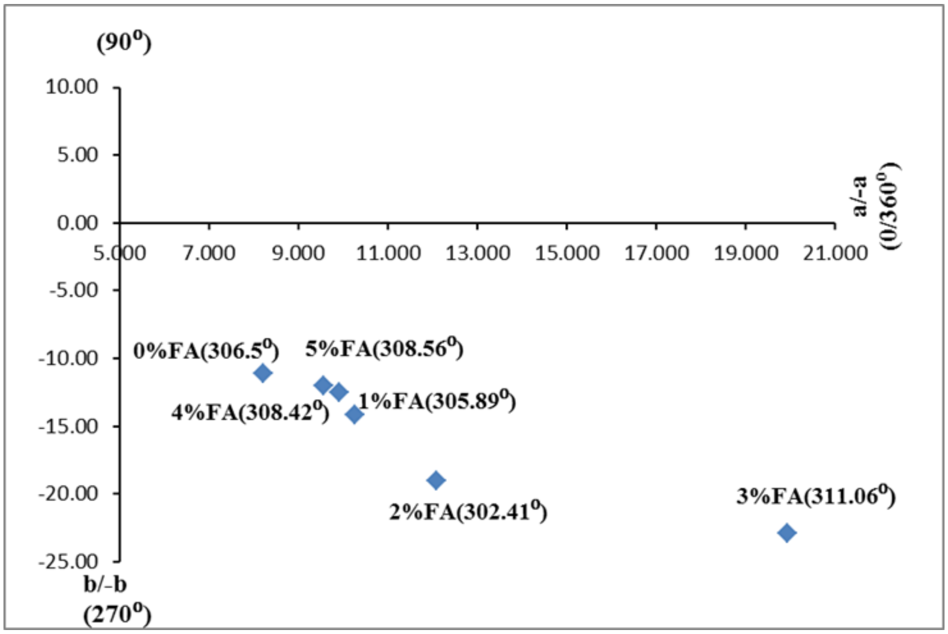


Figure 5.11: Relationship between percentage of FA and C* values (%) for purified *M. malabathricum*-PVA blends FA during 3 month of storage

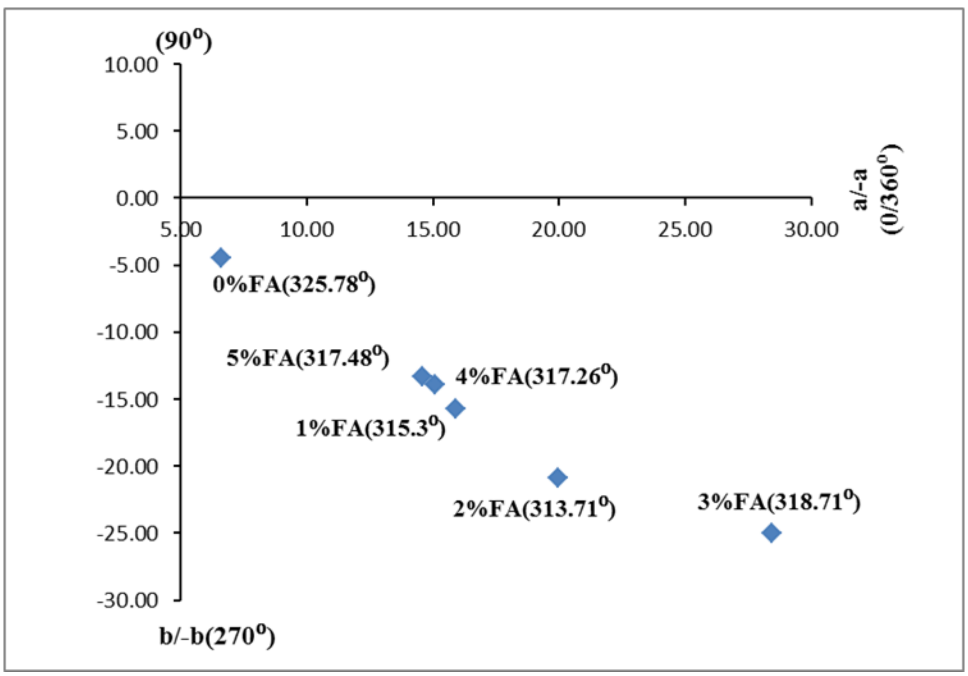
On the other hand, based on the table 5.7 below, it can visibly note that the non-enhanced sample for purified anthocyanin-PVA blends from fruit pulp *M.malabathricum* present in positive a* value (8.213±0.015) and negative b* (-22.871±0.010) with hue angle

(306.500±0.014)at zero time of storage. However, addition of FA significantly enriched the blue colour, with resulted in more negative b* value since b* measures blueness when negative.According to figure 5.12(a) to 5.12(d), it can be realized that addition of 3% FA gave better enhancement with resulted in positive a* value (19.928±0.009) and more negative b* value (-24.050±.0086) with the H^{o} (311.060±0.011).

Moreover, the colour of non-enhance sample (0 % FA) experience decreased in H^{o} to the lower value during 3 month of storage can be noted which means that the colours of the samples was faded as presents in figure 5.10(a) to 5.10(d). It can be realized that, at the end of storage, the a* value decreased to (2.031±0.013) which the lowest value and the b* increased to more yellowness value (8.211±0.013) with the H^{o} (76.106±0.011).Nevertheless, addition of 3% FA resulted in improved the colour stability of purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends by a* value recorded at the end of storage was highest (16.407±0.012) and b* still in negative value (-1.080±0.008) with the H^{o} (356.230±0.010).

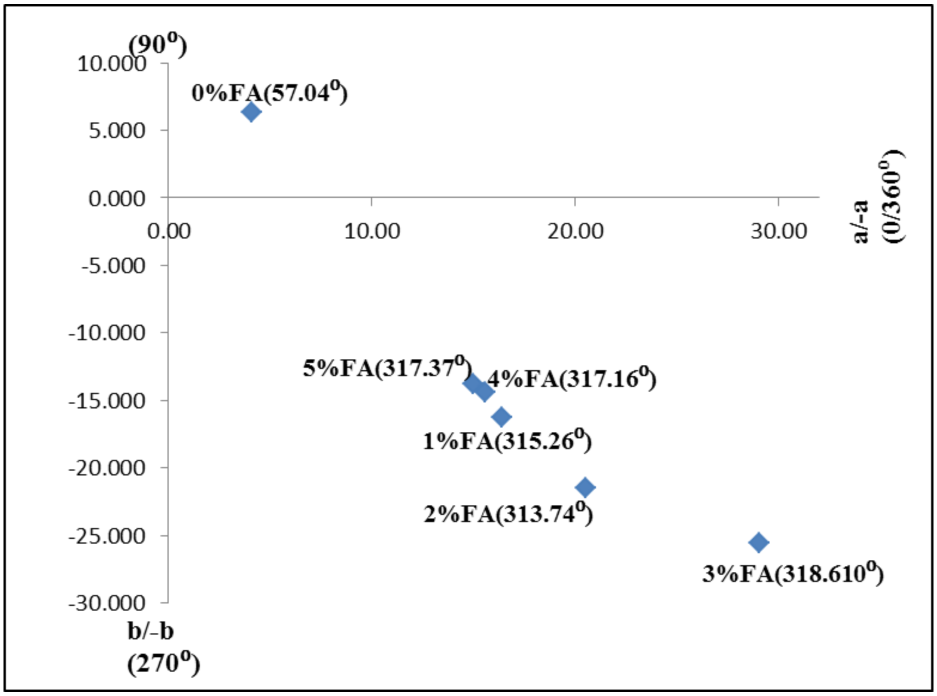


(a)



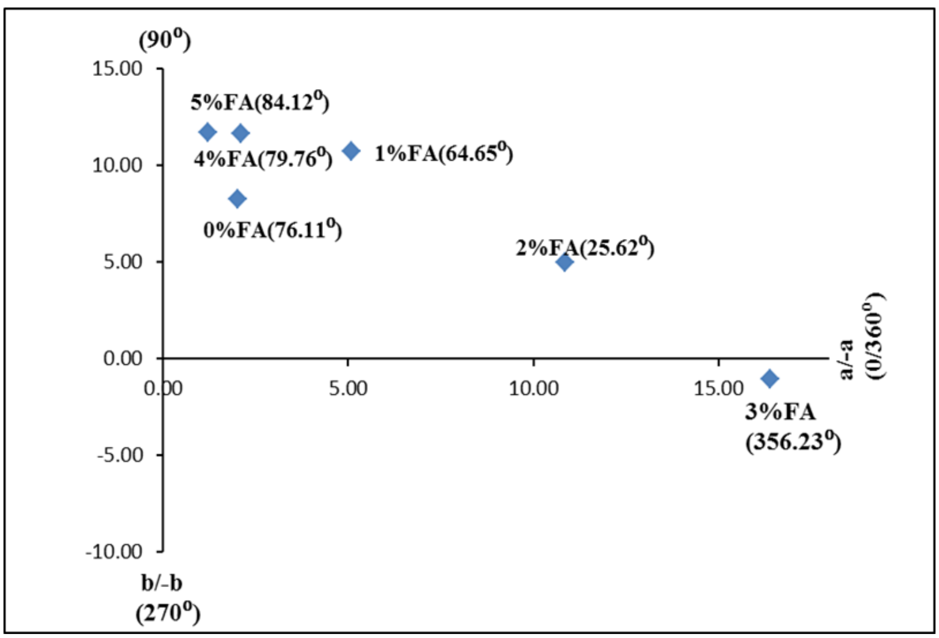
(b)

Figure 5.12: Relationship between percentage of FA and H◦ with a*b* co-ordinate for crude *M. malabathricum*-PVA blends during (a) 0 month, (b) 1 month, (c) 2 month and (d) 3 month of storage



(c)

'Figure 5.12, continued'



(d)

'Figure 5.12, continued'

Table 5.7: Relationship between percentage of FA and L*C* a* and b* values for purified anthocyanin-PVA blends *M. malabathricum*

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
L*	0	0	69.009_{7}	0.012	68.989	69.029
		1	64.32_{11}	0.016	64.296	64.353
		2	60.09_{14}	0.017	60.061	60.119
		3	54.457_{20}	0.012	54.436	54.479
		4	66.337_{9}	0.014	66.313	66.361
		5	68.129_{8}	0.011	68.109	68.148
	1	0	73.282_{5}	0.016	73.255	73.309
		1	56.736_{18}	0.014	56.711	56.761
		2	51.089_{21}	0.018	51.058	51.119
		3	44.682_{23}	0.010	44.665	44.698
		4	59.216_{15}	0.011	59.196	59.235
		5	61.258_{12}	0.016	61.231	61.286
	2	0	73.633_{4}	0.019	73.601	73.666
		1	56.337_{19}	0.015	56.312	56.363
		2	50.598_{22}	0.019	50.564	50.631
		3	44.181_{24}	0.012	44.161	44.202
		4	58.828_{17}	0.016	58.801	58.856
		5	60.896_{13}	0.016	60.868	60.924
	3	0	91.130_{1}	0.015	91.103	91.156
		1	70.814_{6}	0.017	70.785	70.844
		2	65.901_{10}	0.014	65.876	65.926
		3	58.874_{16}	0.008	58.861	58.888
		4	78.433_{3}	0.014	78.409	78.457
		5	83.005_{2}	0.011	82.987	83.024

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.7, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
c*	0	0	13.807_{17}	0.012	13.787	13.828
		1	17.498_{13}	0.018	17.467	17.529
		2	22.527_{7}	0.016	22.499	22.555
		3	30.334_{3}	0.004	30.326	30.341
		4	15.925_{15}	0.017	15.895	15.955
		5	15.342_{16}	0.017	15.313	15.371
	1	0	8.0002_{3}	0.014	7.976	8.023
		1	22.383_{8}	0.015	22.357	22.409
		2	28.906_{5}	0.012	28.886	28.926
		3	37.843_{2}	0.013	37.821	37.865
		4	20.556_{10}	0.019	20.522	20.589
		5	19.7541_{2}	0.018	19.723	19.785
	2	0	7.5262_{4}	0.016	7.498	7.553
		1	23.075_{6}	0.013	23.052	23.098
		2	29.725_{4}	0.012	29.703	29.746
		3	38.734_{1}	0.006	38.723	38.745
		4	21.234_{9}	0.013	21.212	21.256
		5	20.390_{11}	0.019	20.358	20.423
	3	0	8.458_{22}	0.013	8.435	8.481
		1	11.864_{19}	0.019	11.831	11.896
		2	11.956_{18}	0.018	11.925	11.986
		3	16.442_{14}	0.009	16.427	16.457
		4	11.810_{20}	0.014	11.786	11.834
		5	11.751_{21}	0.012	11.729	11.772

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.7, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
h*	0	0	306.500_{16}	0.014	306.476	306.524
		1	305.890_{17}	0.019	305.856	305.923
		2	302.410_{18}	0.014	302.386	302.433
		3	311.060_{13}	0.011	311.042	311.079
		4	308.420_{15}	0.014	308.395	308.444
		5	308.560_{14}	0.017	308.531	308.589
	1	0	325.780_{2}	0.012	325.759	325.801
		1	315.300_{9}	0.014	315.276	315.325
		2	313.710_{12}	0.013	313.687	313.732
		3	318.710_{3}	0.009	318.694	318.726
		4	317.260_{7}	0.016	317.232	317.289
		5	317.480_{5}	0.020	317.446	317.514
	2	0	57.044_{23}	0.016	57.015	57.072
		1	315.260_{10}	0.012	315.239	315.281
		2	313.740_{11}	0.012	313.719	313.762
		3	318.610_{4}	0.013	318.588	318.632
		4	317.160_{8}	0.016	317.133	317.188
		5	317.370_{6}	0.018	317.338	317.402
	3	0	76.106_{21}	0.011	76.087	76.125
		1	64.648_{22}	0.015	64.623	64.674
		2	24.618_{24}	0.012	24.597	24.638
		3	356.230_{1}	0.010	356.213	356.247
		4	79.762_{20}	0.020	79.728	79.796
		5	84.119_{19}	0.019	84.087	84.152

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.7, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	0	8.213_{18}	0.015	8.186	8.239
		1	10.259_{15}	0.016	10.231	10.286
		2	12.077_{13}	0.012	12.056	12.099
		3	19.928_{5}	0.009	19.912	19.943
		4	9.898_{16}	0.015	9.872	9.924
		5	9.564_{17}	0.017	9.534	9.594
	1	0	6.616_{19}	0.010	6.599	6.632
		1	15.91_{28}	0.015	15.885	15.938
		2	19.976_{4}	0.010	19.959	19.993
		3	28.437_{2}	0.012	28.416	28.457
		4	15.100_{10}	0.013	15.078	15.123
		5	14.562_{12}	0.021	14.526	14.599
	2	0	4.0942_{1}	0.014	4.069	4.119
		1	16.393_{7}	0.018	16.362	16.424
		2	20.553_{3}	0.017	20.523	20.583
		3	29.060_{1}	0.007	29.049	29.072
		4	15.57_{19}	0.015	15.545	15.598
		5	15.004_{11}	0.011	14.986	15.023
	3	0	2.031_{23}	0.013	2.008	2.054
		1	5.080_{20}	0.012	5.059	5.102
		2	10.870_{14}	0.011	10.851	10.889
		3	16.407_{6}	0.012	16.386	16.427
		4	2.099_{22}	0.014	2.074	2.124
		5	1.204_{24}	0.019	1.171	1.236

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.7, continued'

CIELab value	Time (month)	FA (%)	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	0	-11.099_{18}	0.014	11.074	11.124
		1	-14.176_{10}	0.017	14.146	14.205
		2	-19.017_{6}	0.013	18.994	19.039
		3	-22.871_{3}	0.010	22.853	22.889
		4	-12.476_{14}	0.010	12.458	12.494
		5	-11.997_{15}	0.014	11.973	12.021
	1	0	-4.498_{23}	0.015	4.472	4.524
		1	-15.742_{8}	0.017	15.713	15.771
		2	-20.893_{5}	0.018	20.862	20.924
		3	-24.969_{2}	0.012	24.949	24.989
		4	-13.949_{11}	0.016	13.921	13.976
		5	-13.349_{13}	0.013	13.327	13.371
	2	0	6.315_{21}	0.015	6.289	6.341
		1	-16.241_{7}	0.018	16.211	16.272
		2	-21.475_{4}	0.014	21.451	21.498
		3	-25.610_{1}	0.008	25.596	25.624
		4	-14.437_{9}	0.009	14.421	14.453
		5	-13.808_{12}	0.012	13.787	13.828
	3	0	8.211_{20}	0.013	8.189	8.234
		1	10.722_{19}	0.014	10.697	10.746
		2	4.981_{22}	0.018	4.951	5.012
		3	1.080_{24}	0.008	1.066	1.095
		4	11.622_{17}	0.015	11.597	11.648
		5	11.690_{16}	0.010	11.672	11.707

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

Table 5.8:Influence of different percentage of FA on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin-PVA blend from *M.malabathricum*

FA (%)	TIME (Month)					
	0	**1**	**2**	**3**	**ΔE_1**	**ΔE_3**
0	s_0=0.2001	s_1=0.1092	s_2=0.1022	s_3=0.0928	ΔE_1=8.024	ΔE_3=30.007
1	s_0=0.2720	s_1=0.3945	s_2=0.4096	s_3=0.1675	ΔE_1=9.592	ΔE_3=26.246
2	s_0=0.3749	s_1=0.5658	s_2=0.5875	s_3=0.1814	ΔE_1=12.121	ΔE_3=24.721
3	s_0=0.5570	s_1=0.8469	s_2=0.8767	s_3=0.2793	ΔE_1=13.128	ΔE_3=22.511
4	s_0=0.2401	s_1=0.3471	s_2=0.3609	s_3=0.1506	ΔE_1=8.941	ΔE_3=28.069
5	s_0=0.2252	s_1=0.3225	s_2=0.3348	s_3=0.1416	ΔE_1=8.611	ΔE_3=29.193

The above result was further analyses in term of Total Colour difference (ΔE) and saturation(s).Table 5.8 displayed the results obtained from the analysis for crude anthocyanin–PVA blend with different addition FA. The saturation (s) is the calculation of an area visualized by an observer which the scalar value derived from the CIELab parameters colour values. This determined as the chromaticity to lightness or the ratio of C* to L*, According to the table, the different percentage of FA gave variation of colour and resulted in different saturation of colour for puriified anthocyanin-PVA blend. Highest colour saturation recorded for the samples at 3% FA added (s_2=0.8767) in the 2nd month of the storage and gradually decreased during storage with the saturation recorded were s_3=0.1814 which the highest saturation recorded compared to the other samples. Consequently these results showed that samples containing 3% FA resulted in more vivid purple-blue colour compared to the other samples studied. Though, as realized in the table the lowest saturation value recorded for the non-enhance samples with the saturation was observed to decreased over the storage period from s_0=0.2001, to s_3 =0.0928.Therefore it directly noticed that, the visual colour of sample of non-enhance samples obviously lightened during the 3 month of the storage.

ΔE represents the colour change of three colour coordinate (C*, L* and h*) of sample before and after exposed to high UV irradiadiation for 3 month. Smallest (ΔE**)** was noticed for the samples with 0% FA, ΔE_1=8.024 at the beginning of the storage. After 3 month of the storage the ΔE_3=30.007 was increased for which showed the colour of samples varied compared to the first month with the saturation only s_3=0.0928. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample with 3% FA (ΔE_1=13.128) which showed that the crude anthocyanin-PVA blends was effectively enhanced**.**Nevertheless at the end of the storage ΔE of 3% FA samples was increased (ΔE_3=22.511) but still most coloured than the others samples tested with the most saturated coloured (s_3=0.2793)

5.3.2. Influence of different pH on Visual Colour Variation of purified anthocyanin-PVA blends

Figure 5.13 displays the influence of different pH (pH initial 5.9, pH 1,3, 7,9 and 11) on visual colour variation for purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends in terms of L* (lightness).It can be noticed that from pH 1 the lightness percentage of purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends (68.487±0.018) decrease over increasing pH 11 (61.039±0.015) at zero time of storage. However, when approach pH 5.9, the lightness started to slightly increase (69.009±0.012) before decrease when pH reaching pH 7 (65.889±0.019) and continue follow the decreasing trend. In addition during the storage period, the lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (88.355±0.0184) after the 3 month of the storage. As expected, the slightly increased in L* value was observed for the sample in acidic region, pH 1, pH 3 and pH 5 with the L* value recorded were (82.889±0.009), (82.269±0.009) and (87.181±0.015) respectively.

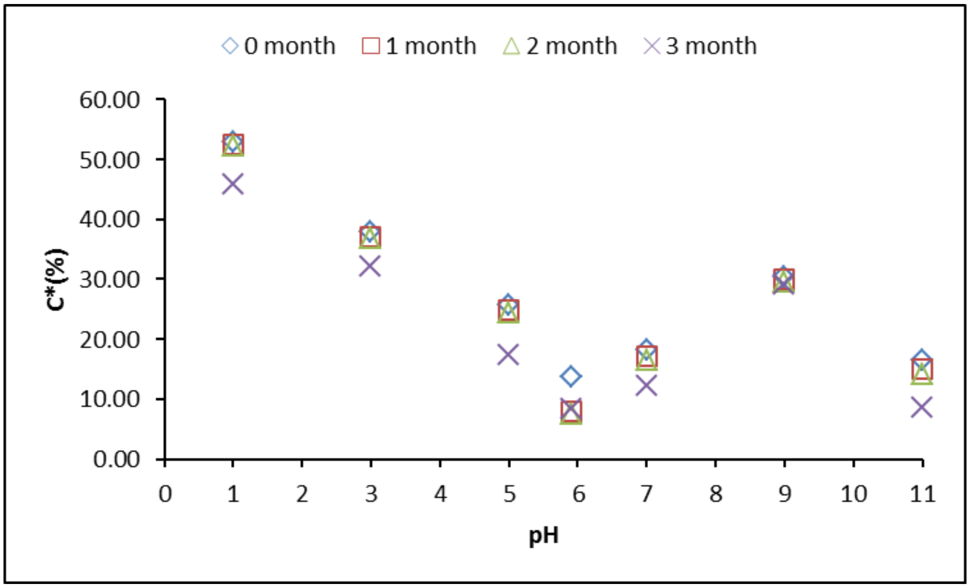


Figure 5.13: Relationship between pH variation and L* values (%) for purified *M. malabathricum*-PVA blends during 3 month of storage

Altered the pH for purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends also gave different chroma value. As seen figure 5.14 the C* value for most acidic purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends, pH1 (52.837±0.014) recorded as the maximum chroma value compared to the other samples studied. Though, from the table It can be realised that, as starting pH 1 the chromaticity decrease (52.837±0.014) with increasing pH 11 (16.406±0.010) but when reaching pH 9 the chromaticity were slightly increase (30.340±0.015).On the hand, the chroma of the purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends also decreased during 3 month of storage which result in dull in colour. As seen in table, most coloured sample pH 1 also experience the colour fading by reducing in C* value over time and it obviously at the end of the storage, the C* value recorded was (45.929±0.009) which still resulted in the highest C* value (more brighter colour) compared to the other pH study. Thus, these results indicates that, variation of pH significantly affect the C* value over the storage period.

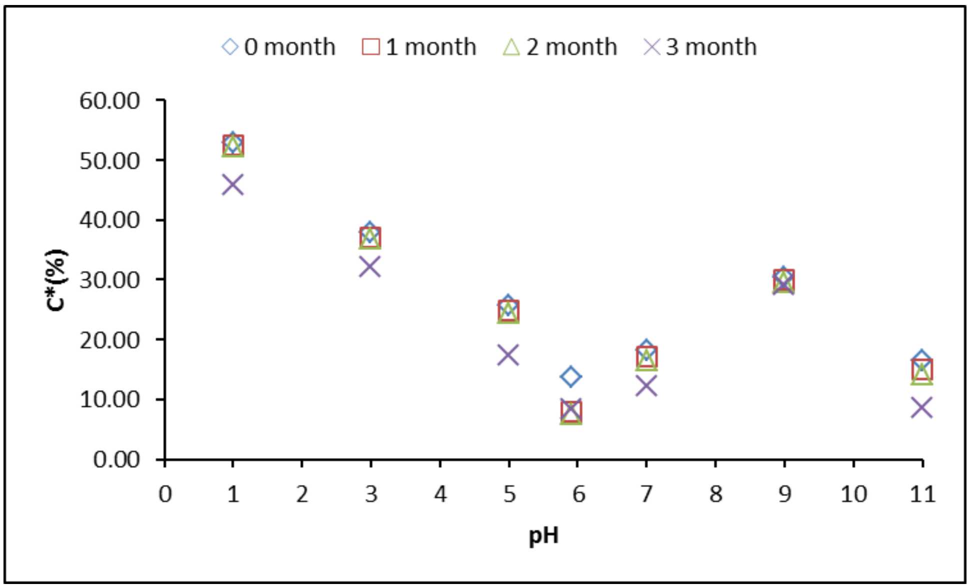
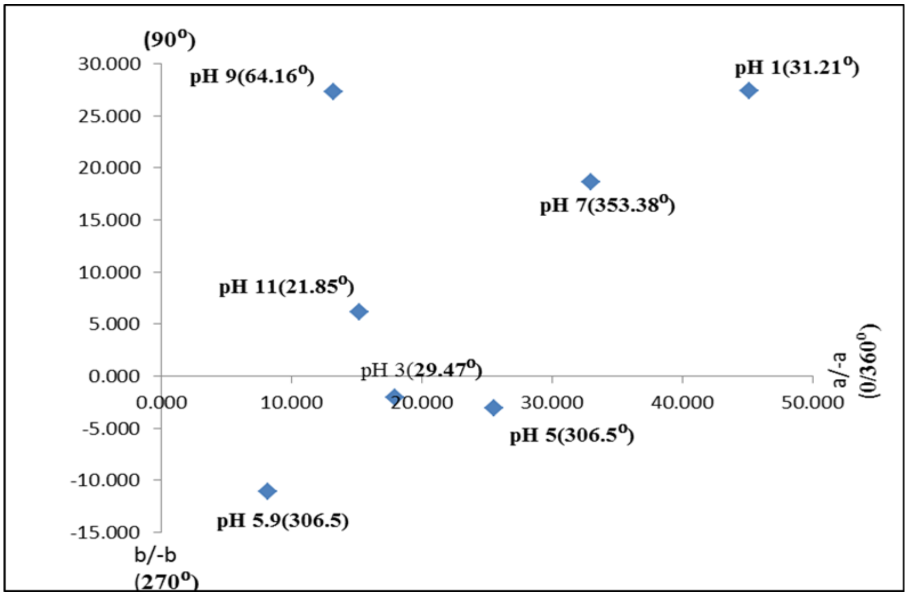


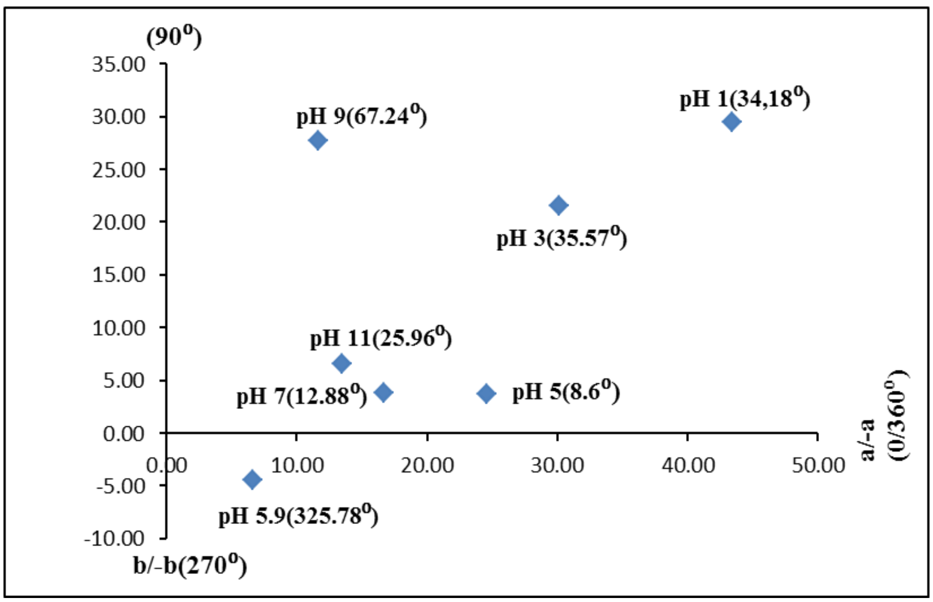
Figure 5.14: Relationship between pH variation and C* values (%) for purified *M. malabathricum*-PVA blends during 3 month of storage

Hue, which also vital affect caused by variation of pH. The detailed results from stastical analysis display in table 5.9. As in table, Further augmentation of pH 1, H^{o} (31.207±0.010) to higher pH 5.8 caused an important counter clockwise shift of hue angle H^{o} (306.500±0.014), meaning that the hues now moved back to yellower tonalities. Based on Figure 5.15(a) to 5.15(d), it shows that the pH 5.8 of purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends present in positive a* values (45.192±.010) and negative b* values (-37.521±.014) with hue angle (h_{ab} 306.500±0.014) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (45.19±0.010) and negative b* values moved to positive value (27.377±0.014) while hue angle moved clockwise to lower value (h_{ab} 31.207±0.010) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (13.222±0.012) and b* slightly increase to positive values (27.308±0.018) while hue angle moved counterclockwise (h_{ab} 64.164±0.012).

Furthermore, the pH variation also affects the visual colour stability of purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends during storage. Based on the table, it clearly noticed that the visual colour of samples with more alkaline pH easily faded during storage with the a* (4.471±.0217) moved to lower positive value and b* slightly moved to more yellower tonalities (7.252±0.017), while the hue angle moved counterclockwise (h_{ab} 68.078±0.0182) which showed the colour degradation of purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends. Additionaly, the most coloured sample at pH 1 also experience calour degradation durig 3 month of the storage with the a* values started to move to the less positive(26.489±0.019) and b* increased to more positive value (37.521±0.014) with increasing the H^o (50.072±.0156). Nonetheless it again can be noticed that, at the end of the storage, the pH 1 succesfully improved the colour stability of the purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends which resulted in the most coloured sample compared to the other pH study.

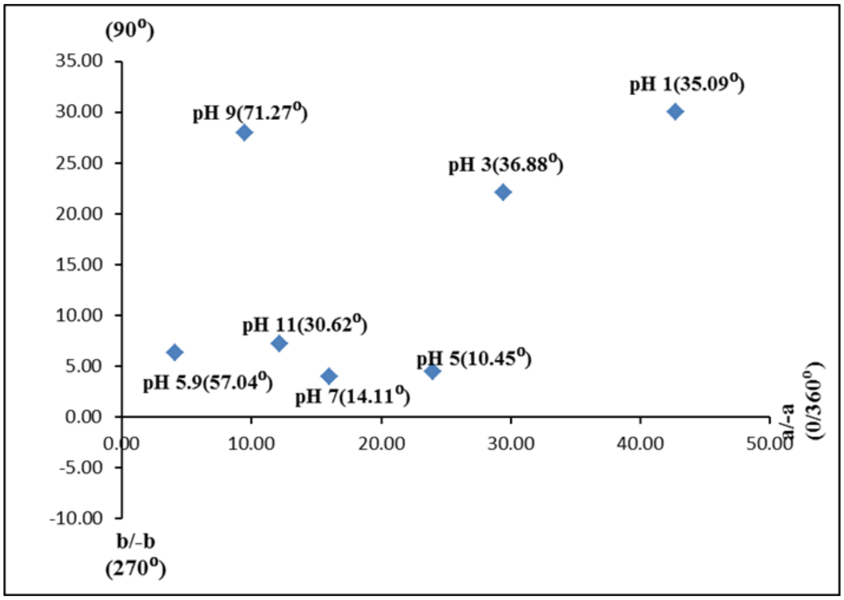


(a)



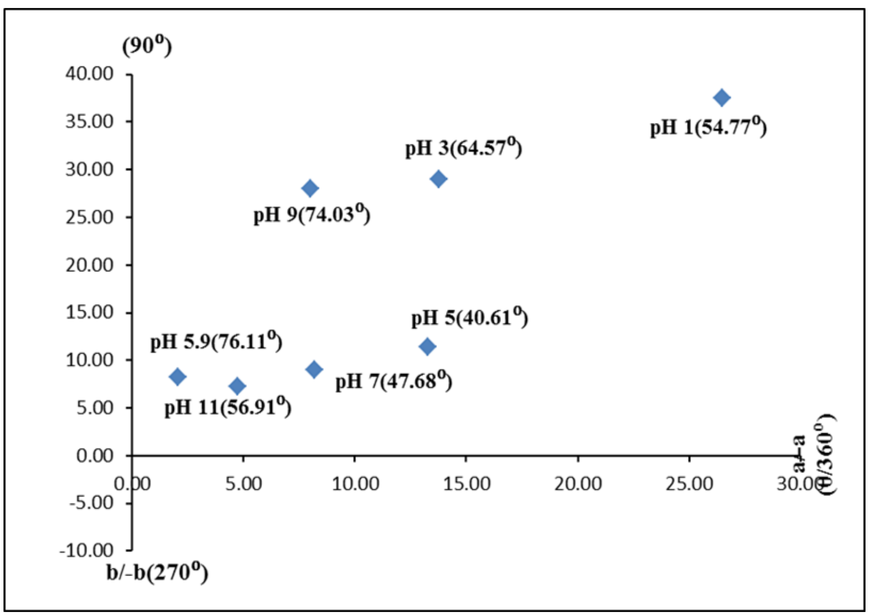
(b)

Figure 5.15: Relationship between pH variation and H◦ with a*b* co-ordinate for purified M. malabathricum-PVA blends during (a) 0 month, (b) 1 month, (c) 2 month and (d) 3 month of storage



(c)

'Figure 5.15, continued'



(d)

'Figure 5.15, continued'

Table 5.9: Relationship between pH variation and L*C* a* and b* values for purified anthocyanin-PVA blends *M.malabathricum*

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
L*	0	1	68.487_{22}	0.018	68.455	68.519
		3	67.171_{23}	0.017	67.141	67.201
		5	65.477_{25}	0.012	65.456	65.499
		5.9	69.009_{21}	0.012	68.989	69.029
		7	65.889_{24}	0.019	65.856	65.921
		9	62.031_{26}	0.017	62.001	62.061
		11	61.039_{27}	0.015	61.012	61.065
	1	1	73.289_{16}	0.014	73.264	73.314
		3	72.312_{18}	0.015	72.286	72.339
		5	69.181_{20}	0.012	69.161	69.201
		5.9	73.282_{16}	0.016	73.255	73.309
		7	76.596_{11}	0.016	76.567	76.624
		9	76.248_{12}	0.016	76.221	76.276
		11	75.862_{13}	0.011	75.843	75.881
	2	1	73.588_{15}	0.012	73.567	73.609
		3	72.621_{17}	0.019	72.588	72.653
		5	69.571_{19}	0.016	69.542	69.599
		5.9	73.633_{14}	0.019	73.601	73.666
		7	76.748_{10}	0.015	76.721	76.774
		9	78.235_{8}	0.014	78.211	78.259
		11	77.860_{9}	0.011	77.840	77.879
	3	1	82.889_{6}	0.009	82.873	82.904
		3	82.269_{7}	0.009	82.254	82.285
		5	87.181_{5}	0.015	87.156	87.207
		5.9	91.130_{4}	0.015	91.103	91.156
		7	96.290_{3}	0.020	96.254	96.325
		9	97.132_{1}	0.012	97.112	97.152
		11	96.353_{2}	0.015	96.326	96.379

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.9, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
C*	0	1	52.837_{1}	0.014	52.813	52.862
		3	37.878_{5}	0.017	37.849	37.907
		5	25.745_{13}	0.014	25.721	25.769
		5.9	13.807_{23}	0.012	13.787	13.828
		7	18.115_{16}	0.016	18.087	18.142
		9	30.340_{9}	0.015	30.315	30.366
		11	16.406_{20}	0.010	16.389	16.424
	1	1	52.449_{2}	0.016	52.421	52.476
		3	37.01_{26}	0.014	36.987	37.037
		5	24.843_{14}	0.013	24.821	24.866
		5.9	8.000_{27}	0.014	7.976	8.023
		7	17.127_{18}	0.018	17.096	17.157
		9	30.026_{10}	0.014	30.001	30.051
		11	15.022_{21}	0.015	14.997	15.048
	2	1	52.196_{3}	0.018	52.165	52.227
		3	36.786_{7}	0.017	36.756	36.816
		5	24.379_{15}	0.014	24.354	24.403
		5.9	7.526_{28}	0.016	7.498	7.553
		7	16.473_{19}	0.014	16.450	16.497
		9	29.489_{11}	0.013	29.467	29.511
		11	14.114_{22}	0.011	14.095	14.132
	3	1	45.929_{4}	0.009	45.913	45.944
		3	32.050_{8}	0.016	32.023	32.078
		5	17.471_{17}	0.016	17.443	17.499
		5.9	8.458_{26}	0.013	8.435	8.481
		7	12.151_{24}	0.015	12.125	12.177
		9	29.151_{12}	0.011	29.132	29.171
		11	8.655_{25}	0.012	8.635	8.675

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.9, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
H*	0	1	31.207_{20}	0.010	31.189	31.225
		3	29.468_{22}	0.015	29.441	29.494
		5	353.210_{2}	0.013	353.187	353.232
		5.9	306.500_{4}	0.014	306.476	306.524
		7	353.380_{1}	0.014	353.355	353.405
		9	64.164_{10}	0.012	64.142	64.185
		11	21.852_{24}	0.016	21.824	21.879
	1	1	34.182_{19}	0.014	34.157	34.207
		3	35.573_{17}	0.014	35.549	35.597
		5	8.614_{28}	0.014	8.589	8.639
		5.9	325.780_{3}	0.012	325.759	325.801
		7	12.884_{26}	0.016	12.855	12.912
		9	67.241_{8}	0.016	67.214	67.269
		11	25.968_{23}	0.016	25.939	25.996
	2	1	35.089_{18}	0.017	35.059	35.119
		3	36.883_{16}	0.017	36.853	36.913
		5	10.447_{27}	0.015	10.421	10.473
		5.9	57.044_{11}	0.016	57.015	57.072
		7	14.110_{25}	0.014	14.087	14.134
		9	71.266_{7}	0.013	71.244	71.288
		11	30.620_{21}	0.012	30.600	30.640
	3	1	54.778_{13}	0.012	54.758	54.799
		3	64.575_{9}	0.011	64.556	64.595
		5	40.617_{15}	0.015	40.591	40.643
		5.9	76.106_{5}	0.011	76.087	76.125
		7	47.688_{14}	0.019	47.656	47.721
		9	74.028_{6}	0.012	74.008	74.048
		11	56.913_{12}	0.012	56.893	56.933

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.9, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	1	45.192_{1}	0.010	45.175	45.210
		3	32.978_{4}	0.015	32.951	33.004
		5	25.565_{8}	0.017	25.535	25.595
		5.9	8.213_{22}	0.015	8.186	8.239
		7	17.995_{11}	0.016	17.967	18.022
		9	13.222_{18}	0.012	13.202	13.242
		11	15.228_{14}	0.014	15.203	15.253
	1	1	43.389_{2}	0.014	43.365	43.412
		3	30.105_{5}	0.010	30.087	30.122
		5	24.563_{9}	0.012	24.542	24.585
		5.9	6.616_{24}	0.010	6.599	6.632
		7	16.696_{12}	0.014	16.671	16.721
		9	11.616_{20}	0.011	11.598	11.635
		11	13.506_{16}	0.010	13.489	13.524
	2	1	42.710_{3}	0.013	42.687	42.733
		3	29.424_{6}	0.013	29.401	29.447
		5	23.975_{10}	0.014	23.951	23.999
		5.9	4.094_{26}	0.014	4.069	4.119
		7	15.976_{13}	0.009	15.961	15.992
		9	9.471_{21}	0.015	9.445	9.496
		11	12.146_{19}	0.019	12.113	12.178
	3	1	26.489_{7}	0.019	26.455	26.522
		3	13.760_{15}	0.014	13.736	13.783
		5	13.262_{17}	0.016	13.234	13.291
		5.9	2.031_{27}	0.013	2.008	2.054
		7	8.180_{22}	0.012	8.159	8.202
		9	8.021_{23}	0.018	7.989	8.053
		11	4.725_{25}	0.016	4.697	4.752

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.9, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	1	27.377_{8}	0.014	27.353	27.401
		3	18.634_{12}	0.013	18.611	18.656
		5	-3.041_{27}	0.015	3.014	3.067
		5.9	-11.099_{14}	0.014	11.074	11.124
		7	-2.0872_{8}	0.013	2.064	2.109
		9	27.308_{9}	0.018	27.277	27.338
		11	6.107_{21}	0.017	6.078	6.137
	1	1	29.468_{3}	0.010	29.451	29.485
		3	21.532_{11}	0.018	21.501	21.564
		5	3.721_{26}	0.014	3.696	3.745
		5.9	-4.498_{22}	0.015	4.472	4.524
		7	3.819_{25}	0.017	3.789	3.848
		9	27.689_{7}	0.015	27.662	27.715
		11	6.578_{19}	0.019	6.546	6.611
	2	1	30.005_{2}	0.011	29.986	30.024
		3	22.079_{10}	0.019	22.046	22.113
		5	4.421_{23}	0.014	4.398	4.445
		5.9	6.315_{20}	0.015	6.289	6.341
		7	4.016_{24}	0.017	3.987	4.045
		9	27.927_{6}	0.017	27.897	27.957
		11	7.189_{18}	0.019	7.156	7.222
	3	1	37.521_{1}	0.014	37.496	37.546
		3	28.947_{4}	0.014	28.923	28.972
		5	11.374_{13}	0.014	11.349	11.399
		5.9	8.211_{16}	0.013	8.189	8.234
		7	8.986_{15}	0.014	8.961	9.011
		9	28.026_{5}	0.021	27.989	28.063
		11	7.252_{17}	0.017	7.223	7.281

(Note: Means with the different subscript numbers are significantly different at P<0.05)

Table 5.10: Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin-PVA blends from *M.malabathricum*

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=0.7715	s_1=0.7156	s_2=0.7093	s_3=0.5541	ΔE_1=5.539	ΔE_3=25.693
pH 3	s_0=0.5639	s_1=0.5118	s_2=0.5065	s_3=0.3896	ΔE_1=6.564	ΔE_3=26.526
pH 5	s_0=0.3932	s_1=0.3591	s_2=0.3504	s_3=0.2004	ΔE_1=7.775	ΔE_3=28.813
pH 5.9	s_0=0.2001	s_1=0.1092	s_2=0.1022	s_3=0.0928	ΔE_1=8.024	ΔE_3=30.007
pH 7	s_0=0.2749	s_1=0.2236	s_2=0.2146	s_3=0.1262	ΔE_1=12.297	ΔE_3=33.815
pH 9	s_0=0.4891	s_1=0.3938	s_2=0.3769	s_3=0.3001	ΔE_1=14.312	ΔE_3=35.491
pH 11	s_0=0.2688	s_1=0.1980	s_2=0.1813	s_3=0.0898	ΔE_1=14.930	ΔE_3=36.861

The above result was further analysed interm of Total Colour difference (**ΔE**) and saturation(s).Table 5.10 display the results obtained from the analysis for purified anthocyanin–PVA blend with variation of pH. According to the table, the different pH variation gave different of colour and resulted in different saturation and ΔE of colour for purified anthocyanin-PVA blend. Smallest (ΔE) was noticed for the samples with pH 1 ΔE_1=5.539 at 1st month of the storage. After 3 month of the storage the ΔE_3=25.693 was increased for pH 1 which showed the colour change compared to the first month. Conferring to the table, it can clearly noticed that the highest ΔE at the first month recorded for the sample at pH 11 (ΔE_1=14.930) and at the end of the storage pH 11 gave the highest total color change compared to the other samples tested (ΔE_3=36.861) which showed the huge colour different.

Highest colour saturation recorded for the samples at pH 1 (s_0=0.7715) and gradually decreased during storage with the saturation recorded were s_1=0.7156, s_2=0.7093, s_3=0.5541 correspondingly which showed that pH 1 resulted in more saturated red colour compared to the other acidic pH. Nonetheless the decreasing of the saturation over 3 month of storage period showed that colour of pH 1 samples also degraded. However, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.2688 to s_3=0.0898 at the end of the storage. Hence it directly perceived that, the colour of sample at pH 11 obviously colourless during the 3 month of the storage.

5.3.3. Influence of different pH on Visual Colour Variation of purified anthocyanin-PVA blends containing 3% FA

Influence of different pH (pH initial 5.8, pH 1, 3, 7, 9 and 11) on visual colour variation for purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends containing 3% FA displays in Table 5.11. Parameter in terms of L* (lightness), C* (chroma), H˚ (hue angle notation h_{ab}), a*/-a* (redness and greenness) and b*/-b* (yellowness and blueness) was observed.It can be seen from the Figure 5.16 that the lightness percentage decreased with increased in pH from pH 1 (58.590±0.007) to pH 11 (49.580±0.011) at zero time of storage. Conversely, when approach pH 5.8, the lightness started to slightly increase (54.457±0.012) before underwent to decreased when pH approach pH 7 (50.826±0.007).

The lightness of the samples at different pH experienced increased in L* with the highest increased recorded for the most alkaline pH, pH 11 (75.883±0.011) after the 3 month of the storage.As accordance to the results obtained, the colour stability of the purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends degraded during the storage period under UVB- irradiation by increasing the the L* value which was resulted in lighter colour and the end of the storage pH 3 presents the most colour sample with the lowest L* value (56.932±0.007)

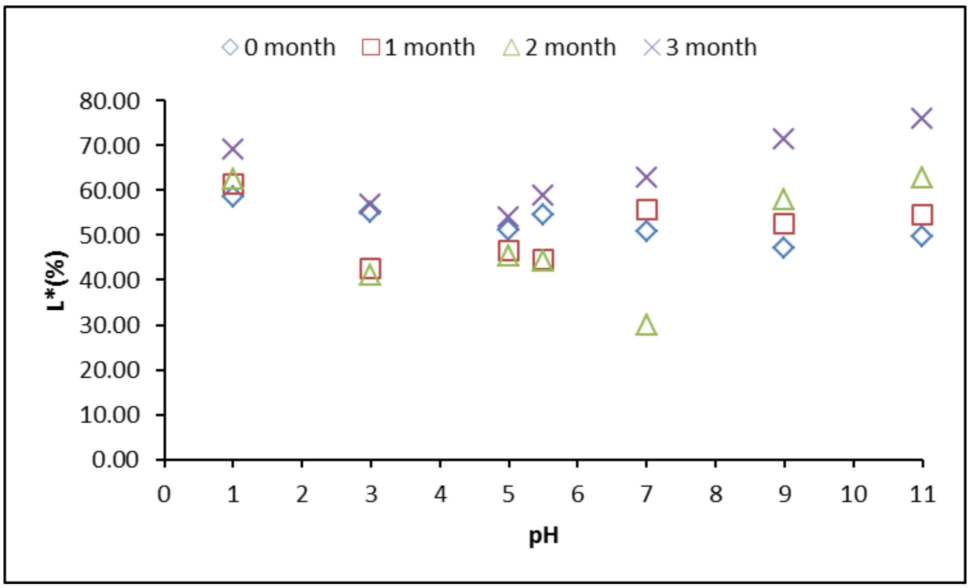


Figure 5.16: Relationship between pH variation and L* values (%) for purified *M. malabathricum*-PVA blends containing 3%FA during 3 month of storage

Furthermore, altered the pH for purified fruit pulp of anthocyanin *M. malabathricum*-PVA blends containing 3% FA also affected the colour chromaticity. As seen in Figure 5.17 the C* value for most acidic crude anthocyanin colourant pH1 (55.306±0.011) recorded as the highest chroma compared to the other sample at the beginning the storage. However, from the table It can be seen as starting pH 1 the chromaticity decrease (55.306±0.011) with increasing pH 11 (19.861±0.012) nevertheless when reaching pH 9 the chromaticity were slightly increase (31.584±0.009).On the hand, the chroma of the purified fruit pulp of anthocyanin *M. Malabathricum*-PVA blends also decreased during 3 month of storage which result in dull in colour. As seen in table, most coloured sample resulted for the sample at pH 3, the C* value recorded was (46.843±0.006) which gave in the highest C* value (brighter colour) compared to the other pH study. The most coloured sample at the beginning of the storage,pH1 did not retained the colour stability which the colour of the samples strongly faded at the end of the storage (33.171±0.010)

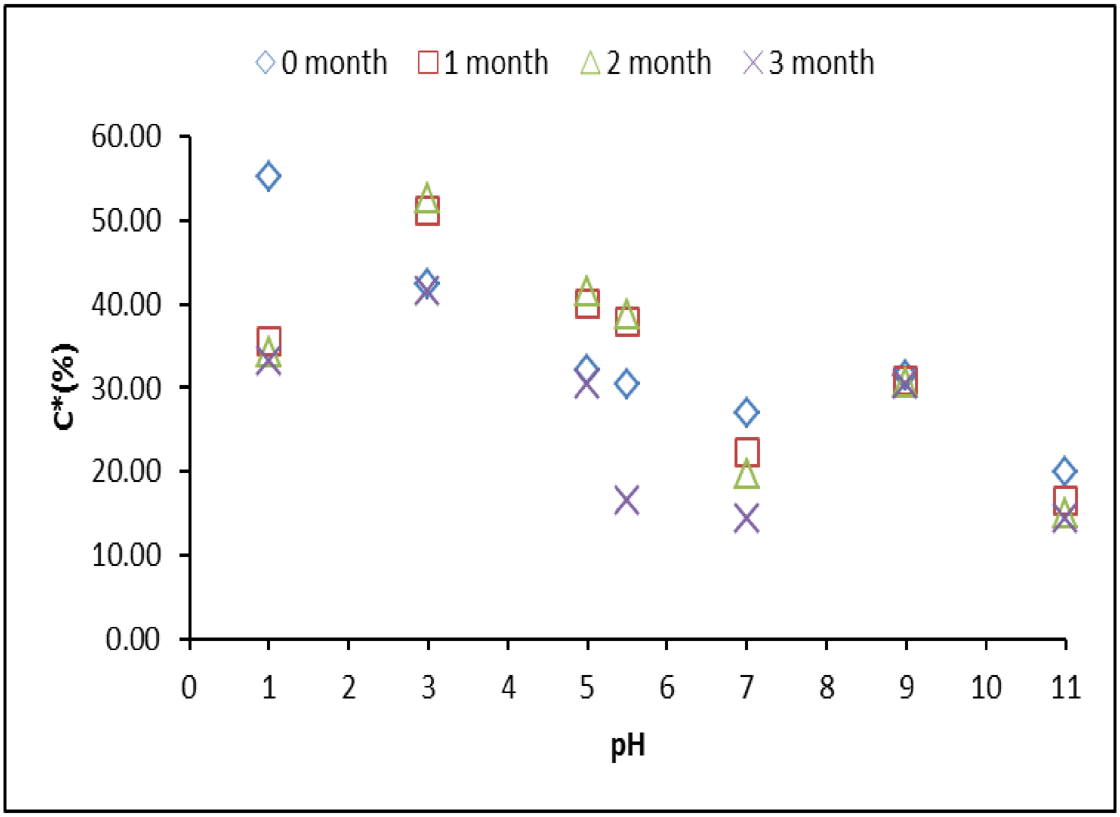
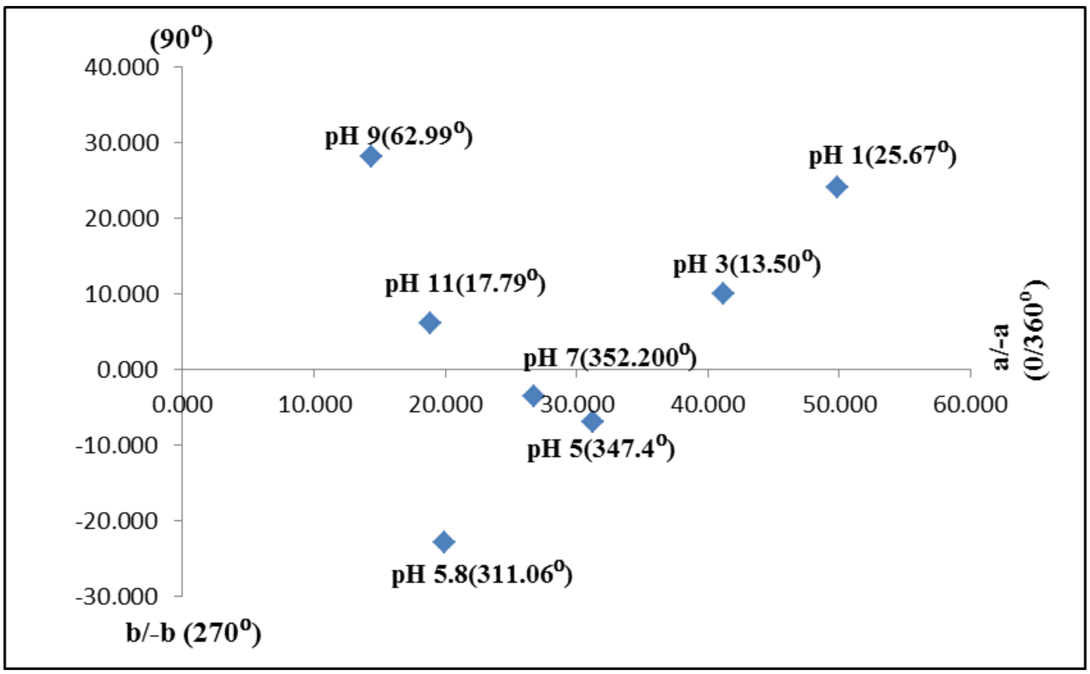


Figure 5.17: Relationship between pH variation and C* values (%) for purified *M. malabathricum*-PVA blends containing 3%FA during 3 month of storage

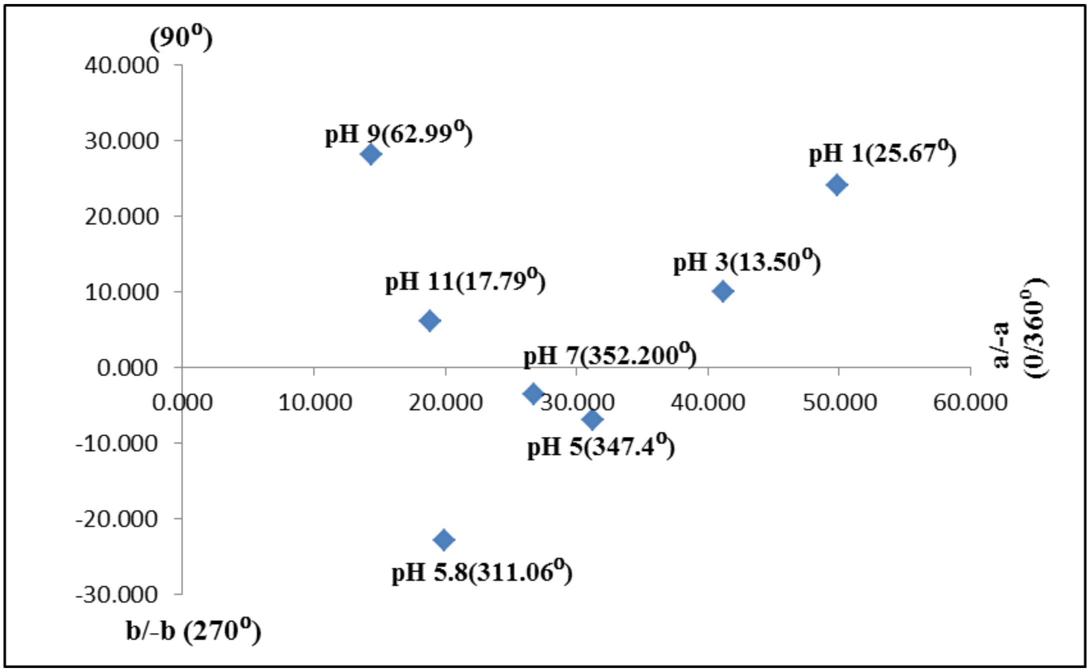
Hue, which also vital affect caused by variation of pH. As in figure 5.18(a) to 5.18(d), further increase of pH 1, H° (25.667±0.012) to higher pH 5.8 initiated an important counterclockwise shift of hue angle H° (311.060±0.011), meaning that the hues now moved back to yellower tonalities. Based on table 6.5, it shows that the pH 5.8 of *Melastoma malabathricum* present in positive a* values (20.111±0.012) and negative b* values (-24.050±0.009) with hue angle H^o (311.060±0.011) which resulted in bluer colour. However, when the pH was adjusted to more acidic, pH 1 the a* value moved to more positiveness (49.849±0.007) and negative b* values moved to positive value (26.217±0.008) while hue angle moved clockwise to lower value (h_{ab} 25.667±0.012) and resulted in more red in colour. Once pH increase to more alkaline region, pH 9 the a* values moved backward to lower positive (14.344±0.008) and b* slightly increase to positive values (33.680±0.010) while hue angle moved counterclockwise H^o (62.990±0.012).

Moreover, the pH variation also affects the visual colour stability of crude anthocyanin colourat during storage. According to the table the highest colour enhancement was observed for the sample with pH 3 with the 1st month increased in hue angle to (342.030±0.007) with a* positively increased (34.972±0.012) with positive b* value moved to negative b* value (-17.153±0.009) and slightly decreased H^o in 2nd month (341.190±0.005) with a* positively increased (49.871±0.008) and negative b* value (-20.958±0.005). pH 3 retained the colours stability after three month of the storage. Based on the table, it clearly noticed that the visual colour of samples with more alkaline pH 11 easily faded during storage with the a* (1.472±0.007) moved to lower positive value and b* slightly moved to more yellower tonalities (13.171±0.007), while the hue angle moved counterclockwise H^o (71.267±0.012) which showed the colour degradation of anthocyanin colourant containing 3% FA. Moreover, In contrast for crude colourant containing 3% FA, the most coloured sample at pH 1 in the beginning of the storage obviously experience calour degradation during 3 month of the storage compared to pH 5 and pH 5.8 with the a* values started to move to the less positive (21.851±0.005) and b* decreased to more positive value (26.258±0.007) with increasing the H^0 (48.796±0.011). Nonetheless it again can be noticed that, at the end of the storage, the pH 3 succesfully improved the colour stability of the crude colourant containing 3% FA which resulted in the most brighter colour compared to the other pH study. Detailed resuls obtained presents in Table 5.11.



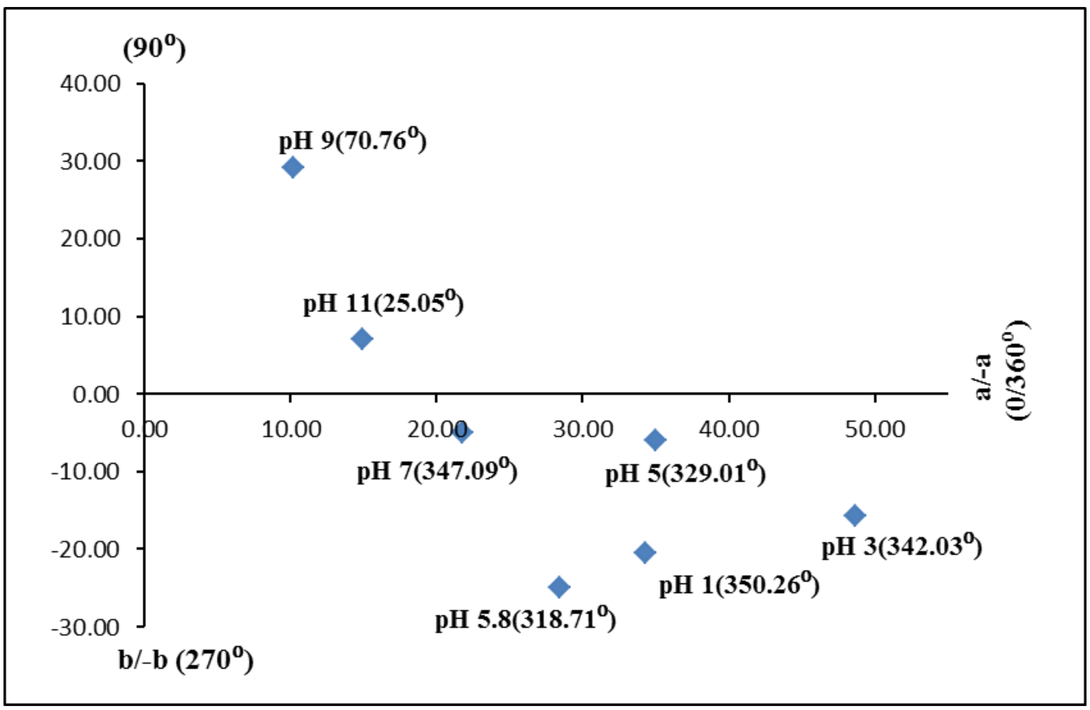
(a)

Figure 5.18: Relationship between pH variation and H◦ with a*b* co-ordinate for purified *M. malabathricum*-PVA blends containing 3%FA during (a) 0 month, (b) 1 month, (c) 2 month and (d) 3 month of storage



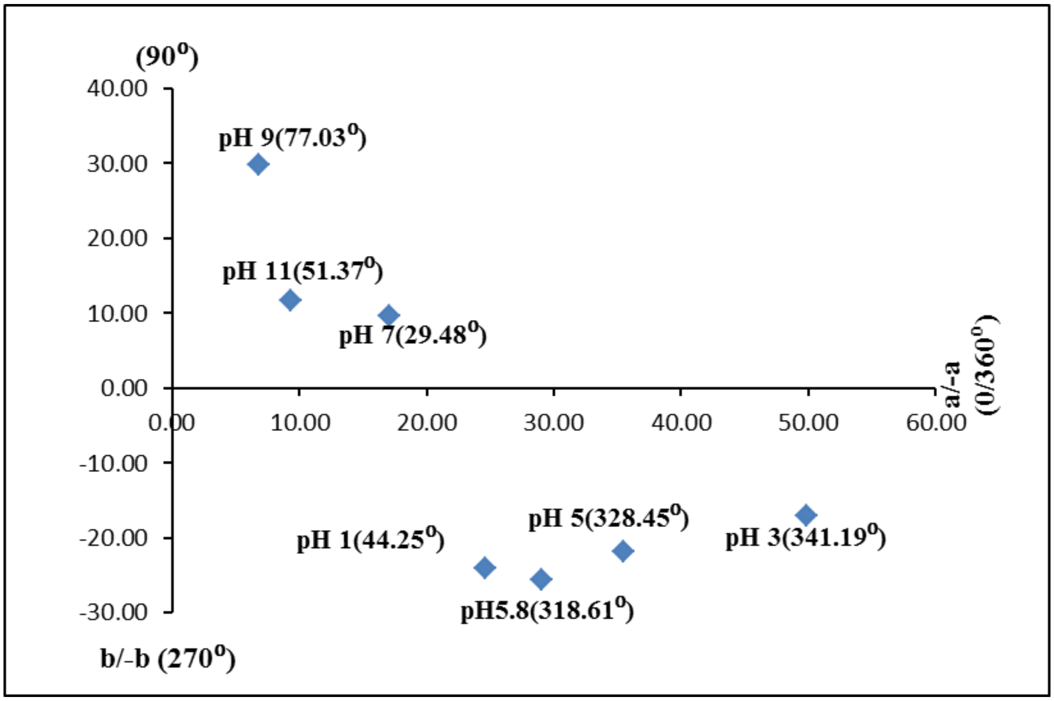
(b)

'Figure 5.18, continued'



(c)

'Figure 5.18, continued'



(d)

'Figure 5.18, continued'

Table 5.11: Relationship between pH variation and L*C* a* and b* values for purified anthocyanin-PVA blends *M. malabathricum* containing 3% FA

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
L*	0	1	58.590_{10}	0.007	58.578	58.601
		3	55.231_{14}	0.006	55.221	55.241
		5	50.992_{15}	0.006	50.981	51.003
		5.8	54.457_{18}	0.012	54.436	54.479
		7	50.826_{21}	0.007	50.814	50.837
		9	47.177_{22}	0.011	47.158	47.196
		11	49.580_{9}	0.011	49.562	49.599
	1	1	61.487_{27}	0.008	61.474	61.501
		3	42.654_{25}	0.007	42.642	42.666
		5	46.614_{23}	0.007	46.602	46.626
		5.8	44.682_{11}	0.010	44.665	44.698
		7	55.749_{17}	0.008	55.735	55.763
		9	52.658_{20}	0.005	52.649	52.667
		11	54.453_{5}	0.012	54.432	54.473
	2	1	62.434_{28}	0.010	62.418	62.451
		3	41.247_{26}	0.006	41.236	41.257
		5	45.293_{24}	0.005	45.284	45.301
		5.8	44.181_{8}	0.012	44.161	44.202
		7	29.957_{13}	0.006	29.947	29.967
		9	58.075_{6}	0.006	58.065	58.085
		11	62.788_{3}	0.014	62.765	62.812
	3	1	69.084_{12}	0.007	69.072	69.097
		3	56.932_{16}	0.007	56.919	56.944
		5	53.893_{7}	0.004	53.887	53.900
		5.8	58.874_{4}	0.008	58.861	58.888
		7	62.827_{2}	0.004	62.819	62.834
		9	71.458_{1}	0.007	71.446	71.470
		11	75.883_{1}	0.011	75.864	75.903

(Note: Means with the different subscript numbers are significantly different at P<0.05)

'Table 5.11,continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
c*	0	1	55.306_{1}	0.011	55.287	55.326
		3	42.339_{7}	0.005	42.329	42.348
		5	32.059_{19}	0.006	32.048	32.070
		5.8	30.334_{16}	0.004	30.326	30.341
		7	27.033_{21}	0.006	27.022	27.044
		9	31.584_{11}	0.009	31.568	31.599
		11	19.861_{22}	0.012	19.841	19.882
	1	1	35.482_{5}	0.007	35.469	35.494
		3	51.165_{4}	0.009	51.149	51.181
		5	39.979_{13}	0.005	39.969	39.988
		5.8	37.843_{6}	0.013	37.821	37.865
		7	22.350_{23}	0.010	22.332	22.367
		9	30.851_{14}	0.013	30.829	30.874
		11	16.462_{24}	0.016	16.434	16.489
	2	1	34.375_{9}	0.008	34.362	34.389
		3	52.683_{2}	0.004	52.676	52.689
		5	41.617_{10}	0.008	41.603	41.631
		5.8	38.734_{3}	0.006	38.723	38.745
		7	19.626_{25}	0.008	19.613	19.639
		9	30.579_{15}	0.010	30.562	30.596
		11	14.938_{26}	0.008	14.924	14.953
	3	1	33.171_{17}	0.010	33.153	33.188
		3	41.658_{8}	0.007	41.646	41.669
		5	30.473_{20}	0.007	30.461	30.486
		5.8	16.442_{18}	0.009	16.427	16.457
		7	14.424_{27}	0.007	14.412	14.435
		9	30.382_{12}	0.007	30.369	30.394
		11	14.326_{28}	0.007	14.314	14.339

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.11, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
h*	0	1	25.667_{24}	0.012	25.646	25.689
		3	13.491_{26}	0.009	13.476	13.506
		5	347.400_{4}	0.006	347.389	347.411
		5.8	311.060_{5}	0.011	311.042	311.079
		7	352.200_{2}	0.011	352.181	352.219
		9	62.990_{16}	0.012	62.970	63.011
		11	17.786_{28}	0.010	17.768	17.804
	1	1	350.260_{1}	0.012	350.240	350.281
		3	342.030_{6}	0.007	342.018	342.043
		5	329.010_{10}	0.007	328.997	329.022
		5.8	318.710_{8}	0.009	318.694	318.726
		7	347.090_{3}	0.012	347.069	347.111
		9	70.758_{15}	0.010	70.741	70.775
		11	25.048_{25}	0.007	25.036	25.059
	2	1	44.245_{20}	0.004	44.238	44.252
		3	341.190_{7}	0.005	341.181	341.199
		5	328.450_{11}	0.007	328.438	328.462
		5.8	318.610_{9}	0.013	318.588	318.632
		7	29.484_{22}	0.009	29.468	29.499
		9	77.032_{9}	0.004	77.025	77.039
		11	51.364_{22}	0.011	51.346	51.383
	3	1	48.796_{13}	0.011	48.777	48.814
		3	18.181_{18}	0.007	18.169	18.193
		5	28.865_{19}	0.006	28.854	28.875
		5.8	356.230_{23}	0.010	356.213	356.247
		7	42.977_{21}	0.009	42.962	42.992
		9	78.988_{27}	0.010	78.971	79.004
		11	71.267_{28}	0.012	71.246	71.287

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.11, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
a*	0	1	49.849_{1}	0.007	49.837	49.861
		3	41.17_{15}	0.006	41.161	41.182
		5	31.288_{14}	0.005	31.279	31.296
		5.8	19.928_{9}	0.009	19.912	19.943
		7	26.784_{15}	0.009	26.768	26.799
		9	14.344_{22}	0.008	14.330	14.359
		11	18.912_{17}	0.011	18.893	18.932
	1	1	34.97_{23}	0.012	34.951	34.993
		3	48.670_{4}	0.007	48.658	48.683
		5	34.276_{12}	0.007	34.263	34.288
		5.8	28.437_{7}	0.012	28.416	28.457
		7	21.786_{19}	0.007	21.775	21.798
		9	10.167_{23}	0.006	10.156	10.178
		11	14.914_{20}	0.010	14.896	14.931
	2	1	24.625_{13}	0.006	24.614	24.636
		3	49.87_{12}	0.008	49.856	49.885
		5	35.468_{10}	0.005	35.459	35.477
		5.8	29.060_{6}	0.007	29.049	29.072
		7	17.085_{21}	0.006	17.075	17.095
		9	6.862_{26}	0.008	6.849	6.875
		11	9.327_{24}	0.013	9.304	9.349
	3	1	21.851_{18}	0.005	21.843	21.859
		3	39.579_{8}	0.009	39.563	39.594
		5	26.687_{16}	0.004	26.679	26.694
		5.8	16.407_{11}	0.012	16.386	16.427
		7	10.553_{25}	0.012	10.532	10.573
		9	5.803_{28}	0.010	5.785	5.821
		11	4.601_{27}	0.012	4.581	4.622

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

'Table 5.11, continued'

CIELab value	Time (month)	pH	Mean	Std. Error (± s.e.)	Minimum	Maximum
b*	0	1	23.956_{7}	0.006	23.945	23.966
		3	9.878_{14}	0.008	9.864	9.892
		5	-6.990_{23}	0.008	6.976	7.003
		5.8	-22.871_{20}	0.010	22.853	22.889
		7	-3.668_{28}	0.011	3.649	3.686
		9	28.140_{4}	0.008	28.127	28.154
		11	6.0672_{6}	0.012	6.046	6.089
	1	1	5.998_{25}	0.010	5.981	6.014
		3	-15.783_{16}	0.007	15.771	15.794
		5	-20.579_{11}	0.006	20.569	20.589
		5.8	-24.969_{9}	0.012	24.949	24.989
		7	-4.991_{27}	0.010	4.973	5.009
		9	29.128_{3}	0.011	29.109	29.147
		11	6.970_{24}	0.016	6.943	6.997
	2	1	23.985_{6}	0.013	23.963	24.007
		3	-16.9841_{12}	0.006	16.974	16.994
		5	-21.772_{10}	0.007	21.761	21.784
		5.8	-25.610_{8}	0.008	25.596	25.624
		7	9.660_{22}	0.013	9.638	9.682
		9	29.800_{2}	0.010	29.783	29.817
		11	11.669_{19}	0.011	11.649	11.688
	3	1	24.957_{5}	0.008	24.942	24.971
		3	12.999_{13}	0.008	12.986	13.012
		5	14.711_{15}	0.010	14.693	14.729
		5.8	1.080_{21}	0.008	1.066	1.095
		7	9.833_{18}	0.014	9.809	9.856
		9	29.823_{1}	0.010	29.806	29.841
		11	13.568_{17}	0.012	13.547	13.588

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

Table 5.12:Influence of different pH on Total Colour difference (ΔE) and Saturation (s) of purified anthocyanin-PVA blends from *M.malabathricum* containing 3 % FA

pH	**TIME (Month)**				ΔE_1	ΔE_3
	0	**1**	**2**	**3**		
pH 1	s_0=0.9439	s_1=0.5771	s_2=0.5506	s_3=0.4801	ΔE_1=33.570	ΔE_3=29.917
pH 3	s_0=0.7666	s_1=1.1995	s_2=1.2773	s_3=0.7317	ΔE_1=29.545	ΔE_3=3.895
pH 5	s_0=0.6287	s_1=0.8577	s_2=0.9188	s_3=0.5654	ΔE_1=14.586	ΔE_3=22.372
pH 5.8	s_0=0.5570	s_1=0.8469	s_2=0.8767	s_3=0.2793	ΔE_1=13.128	ΔE_3=22.511
pH 7	s_0=0.5319	s_1=0.4009	s_2=0.3273	s_3=0.2296	ΔE_1=7.139	ΔE_3=24.285
pH 9	s_0=0.6695	s_1=0.5859	s_2=0.5265	s_3=0.4252	ΔE_1=6.962	ΔE_3=25.794
pH 11	s_0=0.4006	s_1=0.3023	s_2=0.2379	s_3=0.1888	ΔE_1=6.367	ΔE_3=30.869

Table 5.12 displays the Total Colour difference (ΔE) of purified anthocyanin-PVA blends containing 3% FA with different pH (pH initial 5.8, pH 1, 3, 7, 9 and 11).Smallest (ΔE) was noticed for the samples with pH 11 ΔE_1=6.367 at the beginning of the storage. After 3 month of the storage the ΔE_3=30.869 was increased for pH 11 which showed the varied in colour compared to the first month. Based on the table also, it can clearly visible that the highest ΔE at the first month recorded for the sample at pH 1 (ΔE_1=33.570) which showed that purified anthocyanin-PVA blends containing 3% FA was successfully enhanced at the beginning of the storage**.** However at the end of the storage pH 3 samples results in small colour changes (ΔE_3=3.895)

The results gained for this analysis of saturation displayed in table 5.12. Conferring to the table, the pH gave variation of colour and resulted in different saturation of colour when altered the pH (pH initial 5.8, pH 1, 3, 7, 9 and 11) for purified anthocyanin-PVA blends containing 3% FA. From the table above the huge colour variation with the adjusting pH was observed. Highest colour saturation recorded for the 1st month for samples at pH 3 (s_1=1.1995) and continued to increase in the 2nd month (s_2=1.2773) of the storage and gradually decreased during storage with the saturation recorded were s_3=0.7317 which the highest saturation recorded compared to the other samples. Hence these results showed that pH 3 resulted in more saturated purple-blue colour compared to the other pH studied. Yet, as realized in the table the most alkaline samples showed the lowest saturation value with the saturation was observed to decreased over the storage period from s_0=0.4006 to s_3=0.1888 at the end of the storage. Therefore it directly noticed that, the colour of sample at pH 11 obviously lost the colour during the 3 month of the storage.

CHAPTER 6: DISCUSSION

6.1. Discussion

Colourant are materials added to the coating to give colour or resistance properties, camouflaging properties etc. (Tracton, 2006) Synthetic colourant is widely studied because their high performance, however this type of material are toxic and can cause injuries to human. Thus in this work are focused on the study of natural colourant as an alternative to the synthetic colourant. Since the natural colourant not stable, this study concentrates to improve their quality and stability.

The CIElab colour system is the most appropriate tool to study stability and quality of natural colourant both in liquid and solid forms. CIE colour system can be represented by a pair of coordinate a* and b*. As accordance to Wrostald et al (2005), L* C* H* a* and b* describes colour better than absorption / transmission spectroscpopy. L* stands for Lightness. L* can vary between 0 and 100, L*=100 means the colour absolutely white while L* implying 0 absolutely black. Hue angle derived from the a* and b* coordinate. Hue is expressed on a 360^0 grid where 0^0 indicates bluish-red, 90^0 indicates yellow, 180^0 indicates green and 270^0 indicates blue. Hence the more positive value of a* is the greater the redness of the colour and when the colour increases in greenness a* take on more negative value. Likewise, the colour tends to be more yellow when b* becomes more positive and increase in blueness as b* become increasingly negative. Further understands the colour variation, ΔE that denotes colour changes can be calculated from the C*, L* and hand the saturation (s) is another parameters for analysis of colour and is defined at the ratio of C* to L*. In this dissertation colour variation was analysed in terms of s and ΔE.

In these works, liquid and solid samples were subjected to UV-B irradiation for 93 days. The liquid samples can be categorized into two groups, one group is the crude or unpurified anthocyanin solution, while the second is purified anthocyanin solution (obtained after chromatography). These two groups of liquid samples are further derived into FA added anthocyanin colourant and free FA anthocyanin colourant from *M. malabathricum*. The ultimate in is to obtain a dye solution consisting of *M. malabthricum* anthocyanin colourant and FA of the most stabilizing compositions (with respect to present study) and with the best pH condition.

Results obtained from colour stability study of untreated crude and purified anthocyanin from *M.malabathricum* shows that the colourants are unstable and are easily degraded, undergo structural transformation and end up with the loss of colour as indicated by the high L* and low C*. The colour fading in crude and anthocyanin colourant have been quantified by the change in saturation(s) value and ΔE for the 93 days exposure period. At the end of the storage period, the saturation of the *M.malabathricum* for both crude and purified colourants decreased indicating colour fade. The initial colour also experienced a change in ΔE to more yellow tonalities. These results are in agreement with Laleh et al. (2006). Although light is necessary for biosynthesis of anthocyanins,it also accelerates their degradation (Markakis,1982). As Janna et al. (2007) revealed daylight (or short wavelength) and incandescent lamp or long wavelength) effect the degradation of the anthocyanin in different solution. These results are in good agreement with that palamadis and Markakis (1975) and Bakhyaeshi et al. (2006).Between crude and purified anthocyanin, the crude extract exhibited a higher s index compared to the purified extract. This indicates that the natural impurities help to stabilize anthocyanin in the plant. This supported by smaller ΔE for crude anthocyanin colourant.

The application of PVA into the crude and purified anthocyanin-PVA produce a coating when applied on glass slides. The samples showed high L* and low c* over the UV-B exposure period of 93 days. Between the crude and purified extract-PVA coatings, the former showed lower L* and ΔE and higher s and C*. Between the PVA added and the PVA free colourant, the colourant incorporated with PVA showed lower L* and ΔE and higher s and C*.This shows that PVA is a good protective coating material that probably is able to delay colour fade of the anthocyanin colourant from UV-B irradiation.

In terms of CIELab co-ordinate, crude samples have the higher positive in cartesan co-ordinate system. The colour of the crude is redder than the purified colourant as indicated by the more positive a* value. The crude have negative b* value compared to purified. This suggests that the crude extract is bluer than the purified extract of anthocyanin from *M. malabathricum*, this is also for the PVA containing anthocyanin.

Ferulic acid is known as light absorbers and is used in sun tan lotion to block light, so in the effort to protect the anthocyanin colourant FA was added. The addition of Ferulic acid as co-pigment from 1% to 5% FA enhances the colour stability of all samples studied during the 93 days of UV-B irradiation exposure. As expected, as FA increased from 1% to 3% successfully enhance the colour of crude and purified anthocyanin colourant with decreased in the L* value with increasing in C* resulted in more brighter colour After addition of FA the colour saturation,s increased and slightly change in ΔE at the end of storage. The colour of the sample with the addition of FA slightly move to blue region with decreased in b* value and decrease in H^0 value to bluer colour. These results are good agreement with the study conducted by Yawadio et al,. (2007) who found that addition of co-pigment effectively enhances the colour of the samples containing anthocyanin.

From the findings in this work , the 3% FA added crude and purified anthocyanins are the most stable. Since the anthocyanin content is constant, the colour enhancement must be due to the percentage FA added. However, further addition of FA from 4% to 5% FA significantly decreased the colour stability of crude and purified anthocyanin colourant manifested by decreased in colour saturation and increase in ΔE compared to the samples containing 3% FA. These results are supported by Hoshino et al (1980), who claim that when the co-pigment concentration exceeds a certain level, no further colour improvement can be observed. This also in agreement with the study conducted by Setareh et al (2007) which stated that the improvement of colour stability depended on concentration of co-pigment added. This also agrees with other studies (Marcovic et al,. 2005; Marcovic et al., 2000 and Abyari et al,. 2006).

Results obtained for PVA blended both purified and crude anthocyanin with 3% FA successfully enhance the colour of samples by increased in saturation of colour (vivid purple-blue). This enhancement of the colour is due to the interaction of the co-pigmentation with chromophore of the anthocyanin that results in the formation coloured complex. The co-pigment interaction blocks water from attacking on flavilium cation. According to Gauche et al. (2010) the complexion of co-pigment with the anthocyanin cause the colour enhancement.

As reported by Ochoa et al., (2001) found that the samples kept by the present of light effectively destroyed however the addition of co-pigment increased the stability the sample. Abyari and others (2006) also mentioned in their study that the UV-irradiation degradation can be avoided by co-pigmentation with some phenolics acid. Setareh and others (2007) reported that the presence of co-pigments in the anthocyanin solution prevented the degradation effect of UV-irradiation over a period of time on anthocyanin pigments significantly. Thus, it obviously inferred that co-pigmentation is the main factor in enhanced the stability of anthocyanin colourant for this study. The addition of Ferulic Acid (FA) as a co-pigment improved the stability of anthocyanin from fruit pulp of *M. malabathricum* by enhancing the anthocyanin colour saturation through 93 days. This result similar with Abyari et al. (2006) which also reported the similar finding which addition of phenolic acid improved the colour stability. This finding also with the good agreement with Baranac et al. (1997) and Gauche et al. (2010). This is because addition of co-pigment significantly stabilized the structure of the anthocyanin. (Marcovic et al,. 2000 and Abyari et al,. 2006).

The colours of all samples in this study decreased after exposed to the high UVB-irradation. The gradual degradation of red colour and blue colour visually observed by similar decreased in a* and increased in b* value.Nevertheless the results gained for this investigation obviously showed that the colour of treated samples with FA also faded during the storage under UVB-irradiation for 3 month with decreasing in their saturation at the end of the storage. This is because as discussed earlier the UVB-irradiation effectively destroyed the anthocyanin which resulted in color loss as reported by Laleh (2006).

In addition from the observation for this study, it can be predicted that as the pH was increase the colour was spreading caused by an important loss of saturation and increased of lightness together with hue shifts (to bluer or yellow tonalities) and resulted in less saturated colour and increased in ΔE. Furthermore, anthocyanin which used as natural colourant in this research are known to display a huge variety of colour variation in the pH ranges from 1-11. This is due to the ionic nature of anthocyanins which enables the changes of the molecule structure according to the prevailing pH, thus resulting in different colours and hues at different pH values (Brouillard 1982; Von Elbe et al., 1996)

Both of crude and purified anthocyanin studied in this research shown the similar result. The chroma result for *M.malabatricum* illustrates the trend, in which the chroma level decrease when the lightness increased over the pH. This result show correlation Gonnet (1999) which stated that the increase the pH resulted in high L* value with decline in C* value coupled with the shift of hue angle. A further increased in pH caused an important shift of hue angle to yellower tonalities. This result correlated with this study which also found the similar tendency. The pH value was important when determining the colour of *M. malabatrichum* .This is because anthocyanins can be found in different chemical forms which depend on the pH of the solution (Kennedy et al., 2000). pH 1 resulted in higher stability compared to the other pH at different condition studied for crude and purified colourant. The relative amount of this structure equilibrium is varied and depends on the pH and anthocyanin structure (spayd et al.,2002; Bakhshyaeshi et al., 2006). This study have correlated with Bakhshyaeshi et al. (2006) which found that increasing pH effectively increased the destruction of anthocyanin and pH 1 is the most stable. This is because flavylium salts are stable only in highly acidic condition and loose the proton at higher pH condition and immediately bound to water to form colourless compound.

At pH 1, the flavylium cation (red colour) is the predominant species and contributes to purple and red colours while at pH values between 2 and 4, the quinoidal blue species are predominant whereas at pH values between 5 and 6 only two colourless species can be observed, which are a carbinol pseudobase and a chalcone. Meanwhile, at pH values higher than 7, the anthocyaninsare degraded depending on their substituent groups due to the presence of unstable blue quinonoidal structures. These statements are correlated with Mazza and Miniati,1993 as below pH 2, anthocyanins were primarily in the form of the red flavylium cation. When pH increase >2, there were rapid proton losses favouring red or blue quinonoidal form. The flavylium cations become hydrated to yield the colourless carbinol or pseudobase, which equilibrated to the open chalcone form, also colourless

Besides that increased the pH from pH 1 to alkaline pH (pH 11) for anthocyanin-PVA blend obviously resulting in fading the colour of samples. This is because, according to Delpech (2000) higher pH caused the degradation rates increased by fading the colours of samples. Angella and little (1997) also found the similar results which state that the destruction of anthocyanin increased by raising the pH. Furthermore Salinas et al. (2003) and Cortes et al. (2006) stated that anthocyanin more stable at an acid pH, however the stability going to decrease at neutral pH and completely destroyed at alkaline pH because at alkaline pH the flavy cation begin to hydrates resulted in calorless carbinol or pseudobase, in equilibrium with the open form of chalcone, which also colourless. Brouillard (1982) and von Elbe and Schwartz (1996) stated that anthocyanins exhibit greater stability under acidic condition at low pH values rather than in alkaline solutions with high pH values.However, several studies demonstrated that some anthocyanins showed an improvement of color stability in the alkaline region around pH 8-9 (Cabrita et al,.2000; Tantituvanont et al.2008)

Based on the results obtained from this research also, pH plays vital factors in enhance the colour of crude and purified anthocyanin complex containing 3% FA stability which pH 3 gave better enhancement by increased the saturation of the colour during the storage which resulted in darker colour (lower L* value) and enhance the colour by (higher C* value) with the shift the hue angle to more bluer colour. Hence this phenomena of co-pigmentation result in the shift to the bluer colour. This show good agreement with Gonnet (1999) which state that addition of co-pigment in pH solution results in lower L* value and high C* value. Furthermore, as observed in this study of colour variation, at pH 3 the colour of the samples tested resulted in more intense bluish colour with resulted increased in saturation and ΔE which showed that the best co-pigmentation effect. This result showed better correlation with Abyari et al. (2006) and Setareh et al. (2007) which indicated that the optimum pH the co-pigmentation of anthocyanins is between approximately pH 3 and 5. This results also range for good agreement with Gauche et al. (2010) which found that pH for the best co-pigmentation effect is around pH 3 where anthocyanins exist essentially as colorless hemiacetals formed due to the hydration of the flavylium cation. As stated by Abyari et al. (2006) at pH 3 without the addition co-pigment resulted in important colour loss, thus addition of co-pigment help in reduce the production of colourless carbinol.

At pH 4-6 the solutions containing only anthocyanin and co-pigment are still coloured. In this pH range quinoidal based are formed and again, the colour retention is due to decrease in the amount of carbinol pseudobase in the solution (Brouillard et al., 1991 and Setareh et al,.2007). While at pH1-2 for sample containing co-pigment the shift of colour is based on interaction of anthocyanidin flavynium cation with the co-pigment. (Setareh et al., 2007). In addition Bakowska et al. (2003) reported that co-pigment effect will occur from the pH value near 1 to neutrality. In contrast with this study reported by Gauche et al 2010 which noted that ferulic acids at pH 1.0 and 2.0, only showed an increase in saturation of colour but not stable.

Gauche et al. (2010) reported that the color of anthocyanins is either orange-red or bluish, depending on the structure. The majority of the naturally occurring anthocyanins, likely hydroxyphenyl pyranoanthocyanins, vitisins, and vinylflavanolpyranoanthocyanins possess a hypsochromically shifted maximum of absorption resulting in orange hue

In addition, Gauche et al. (2010) also found that the use of organic acids and the consequent variation in pH values caused changes in stability of anthocyanins from Cabernet Sauvignon (*Vitis vinifera L.*) grape crude extract with increased half-life time ($t_{1/2}$) of anthocyanins red color after tannic and gallic acids addition were observed at practically all pH values. This result correlated with this study which showed that addition of organic acid (FA) at pH 3 resulted in highest stability compared to pH and organic acid alone.

CHAPTER 7: CONCLUSION AND SUGGESTION FOR FURTHER WORKS

7.1. Conclusion

CIE colour analysis can be used to observe the calour stability of crude and purified natural colourant. This is because CIE colour system really reports on the variations as these are perceived by the human eye. Thus, colours can be precisely described using CIELab colour co-ordinates L* a* b* C* H* and further analyzed in term of *s* and ΔE. Hence for the stability study using CIE system, the crude anthocyanin showed better colour stability during 93 days of storage. The colour faded over the storage period by decreasing in saturation of colour and change in ΔE. Crude *M.malabathricum* colourant showed better stability towards UVB-Iradiation, at pH 3.0 with the addition of 3% FA as co-pigment compared to the purified anthocyanin from fruit pulp of *M.malabatricum*. The loss of colour at the end of storage show significant effect of UVB-irradiiation

Thus, it gave advantage because to extract the purified colourant needs higher cost and limitation in time consuming. Nevertheless the stability of crude anthocyanin studied better at pH 1 without the addition of FA. Crude anthocyanin colourant is more coloured at pH 1 which gave high saturation value. Moreover, the untreated colourant easily to degrade both at all pH studied during storages time. However, crude anthocyanin-PVA blend were slightly increased in stability towards UVB-irradiation. Thus it showed that PVA suitable to be used as a binder in a coating system since the colour intensity and variation was successfully enhanced.

An anthocyanin-containing colourant was extracted from the fruit pulp of *M.malabatrichum* was found to be most coloured at pH 1 and the colour variation are definitely influenced by pH and different percentage of FA added. Addition of 3% FA at pH 3 successfully enhanced the colour of samples study which gave intense colour. Co-pigmentation with addition of FA is a solution phenomenon in which improved the stability of natural calourant. In addition when blended the anthocyanin-containing 3% FA with PVA, the CIELAB results obtained revealed that the colour was not really influenced by addition of polymer, thus make it suitable for the production of natural coloured polymer. As a conclusion, the CIELAB measurements are effective tools in describing colour properties since it indistinctly covered simultaneous or alternate variations of two attributes of colour, lightness and chroma which lead to influential in the appreciation of products' colours.

Overall analysis of calour stability for this study showed that the best stability of crude colourant and purified anthocyanin of *M.malabathricum*-PVA improved by addition of 3% FA at pH 3.For further works, it is suggested to use another UV-absorber in order to improve the stability of coating system towards UV-B irradiation. Besides that, we could look into the use of individual anthocyanin colourant by isolation process and further test with High Performance Liquid Chromatography (HPLC). Besides that, since natural colourant have many benefit and less toxic compare to synthetic colourant, the look into another source of natural colourant are necessary. Besides, it is suggested also to use natural binder combine with natural colourant in order to produce natural water borne coating.

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APPENDIX A

Statiscal output (SPSS) of CIE analysis colourant for Lightnest,L* (Duncan test)

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	15	3	24																							
	9	3		23																						
	14	3			22																					
	8	3				21																				
	3	3					20																			
	13	3						19																		
	7	3							18																	
	2	3								17																
	21	3									16															
	16	3										15														
	10	3											14													
	17	3												13												
	11	3													12											
	1	3														11										
	4	3															10									
	20	3																9								
	5	3																	8							
	0	3																		7						
	6	3																			6					
	12	3																				5				
	19	3																					4			
	22	3																						3		
	23	3																							2	
	18	3																								1
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX B

Duncan test output of CIE colour analysis for C* using SPSS

	FA	N	Subset for alpha = 0.05																					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Duncan[a]	18	3	24																					
	12	3		23																				
	6	3			22																			
	0	3				21																		
	23	3					20																	
	5	3					20																	
	22	3						19																
	4	3							18															
	19	3								17														
	1	3									16													
	11	3										15												
	10	3											14											
	17	3												13										
	20	3													12									
	16	3													12									
	7	3														11								
	2	3															10							
	13	3																9						
	21	3																	8					
	3	3																		7				
	8	3																			6			
	14	3																				5		
	9	3																					4	
	15	3																						3
	Sig.		1	1	1	1	0.1	1	1	1	1	1	1	1	0.3	1	1	1	1	1	1	1	1	1

APPENDIX C

Duncan test output of CIE colour analysis for H* using SPSS

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	6	3	13																							
	12	3		16																						
	21	3			23																					
	20	3				36																				
	19	3					54																			
	22	3						66																		
	23	3							74																	
	18	3								83																
	15	3									325															
	9	3										325														
	14	3											326													
	8	3												327												
	13	3													330											
	7	3														330										
	16	3															333									
	10	3																333								
	17	3																	334							
	11	3																		334						
	3	3																			340					
	2	3																				343				
	1	3																					346			
	4	3																						349		
	5	3																							351	
	0	3																								352
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX D

Duncan test output of CIE colour analysis for a* using SPSS

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	18	3	2																							
	23	3		5.8																						
	22	3			8.7																					
	19	3				13																				
	12	3					17																			
	6	3						17																		
	0	3							18																	
	5	3								21																
	20	3									21															
	4	3										21														
	1	3											22													
	11	3												22												
	10	3													22											
	17	3														23										
	7	3															23									
	16	3																23								
	13	3																	24							
	21	3																		25						
	2	3																			26					
	8	3																				27				
	14	3																					28			
	3	3																						30		
	9	3																							32	
	15	3																								33
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX E

Duncan test output of CIE colour analysis for b* using SPSS

	FA	N	Subset for alpha = 0.05																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Duncan[a]	0	3	2.4																						
	5	3		3.4																					
	4	3			4																				
	6	3				4																			
	12	3					4.7																		
	1	3						5.6																	
	2	3							7.9																
	11	3								11															
	21	3									11														
	3	3										11													
	17	3											11												
	10	3											11												
	16	3												12											
	7	3													13										
	13	3														14									
	20	3															15								
	18	3																17							
	19	3																	18						
	8	3																		18					
	14	3																			19				
	22	3																				20			
	23	3																					20		
	9	3																						22	
	15	3																							23
	Sig.		1	1	1	1	1	1	1	1	1	1	0.5	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX F

Duncan test output of CIE colour analysis for L* using SPSS (PVA)

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	15	3	44																							
	9	3		45																						
	14	3			51																					
	8	3				51																				
	3	3					54																			
	13	3						56																		
	7	3							57																	
	16	3								59																
	21	3									59															
	10	3										59														
	2	3											60													
	17	3												61												
	11	3													61											
	1	3														64										
	20	3															66									
	4	3																66								
	5	3																	68							
	0	3																		69						
	19	3																			71					
	6	3																				73				
	12	3																					74			
	22	3																						78		
	23	3																							83	
	18	3																								91
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX G

Duncan test output of CIE colour analysis for C* using SPSS (PVA)

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	15	3	44																							
	9	3		45																						
	14	3			51																					
	8	3				51																				
	3	3					54																			
	13	3						56																		
	7	3							57																	
	16	3								59																
	21	3									59															
	10	3										59														
	2	3											60													
	17	3												61												
	11	3													61											
	1	3														64										
	20	3															66									
	4	3																66								
	5	3																	68							
	0	3																		69						
	19	3																			71					
	6	3																				73				
	12	3																					74			
	22	3																						78		
	23	3																							83	
	18	3																								91
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX H

Duncan test output of CIE colour analysis for H* using SPSS (PVA)

	FA	N	Subset for alpha = 0.05																					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Duncan[a]	20	3	25																					
	12	3		57																				
	19	3			65																			
	18	3				76																		
	22	3					80																	
	23	3						84																
	2	3							302															
	1	3								306														
	0	3									307													
	4	3										308												
	5	3											309											
	3	3												311										
	8	3													314									
	14	3													314									
	13	3														315								
	7	3														315								
	16	3															317							
	10	3																317						
	17	3																	317					
	11	3																		317				
	15	3																			319			
	9	3																				319		
	6	3																					326	
	21	3																						356
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	0.1	0.1	1	1	1	1	1	1	1	1

APPENDIX I

Duncan test output of CIE colour analysis for a* using SPSS (PVA)

	FA	N	Subset for alpha = 0.05																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Duncan[a]	23	3	1.2																						
	18	3		2																					
	22	3			2.1																				
	12	3				4																			
	19	3					5.1																		
	6	3						6.6																	
	0	3							8.2																
	5	3								9.6															
	4	3									9.9														
	1	3										10													
	20	3											11												
	2	3												12											
	11	3													15										
	17	3														15									
	10	3															15								
	16	3																16							
	7	3																	16						
	13	3																		16					
	21	3																		16					
	3	3																			20				
	8	3																				20			
	14	3																					21		
	9	3																						28	
	15	3																							29
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.5	1	1	1	1	1

APPENDIX J

Duncan test output of CIE colour analysis for b* using SPSS (PVA)

	FA	N	Subset for alpha = 0.05																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	21	3	1.1																							
	6	3		4.5																						
	20	3			5																					
	12	3				6																				
	18	3					8.2																			
	19	3						11																		
	0	3							11																	
	22	3								12																
	23	3									12															
	5	3										12														
	4	3											12													
	11	3												13												
	17	3													14											
	10	3														14										
	1	3															14									
	16	3																14								
	7	3																	16							
	13	3																		16						
	2	3																			19					
	8	3																				21				
	14	3																					21			
	3	3																						23		
	9	3																							25	
	15	3																								26
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX K

Publication of works.

N. Aziz, N.A. Mat Nor, A.F. Mohd-Adnan, R.M. Taha, A.K. Arof, (2012),"Study of anthocyanin stability derived from the fruit pulp of *Melastoma malabathricum* in a coating system", Pigment & Resin Technology, Vol. 41 Iss: 4 pp. 223 – 229 (ISI- published)

A.F. Mohd-Adnan, N.A. Mat Nor, N. Aziz, R.M. Taha, (2011)," Colour Analysis of potential natural colourant from *ixora siemensis* and *Melastoma malabathricum* ", Materials Research Innovations (ISI), Vol. 15 pp. 176 – 183 (ISI-published)

N.A. Mat Nor, N. Aziz, A.F. Mohd-Adnan, R.M. Taha, A.K. Arof, (2012)," Effect of UV-B Irradiation on Polyvinyl Alcohol and *ixora siemensis* Anthocyanins-coated glass", Pigment & Resin Technology, Vol. 42 Iss: 3 (ISI- Waiting for publication)

Pigment & Resin Technology

Emerald Article: Study of anthocyanin stability derived from the fruit pulp of Melastoma malabathricum in a coating system

N. Aziz, N.A. Mat Nor, A.F. Mohd-Adnan, R.M. Taha, A.K. Arof

Study of anthocyanin stability derived from the fruit pulp of *Melastoma malabathricum* in a coating system

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Abstract

Purpose – The purpose of this paper is to evaluate the stability of anthocyanin colorant with and without ferulic acid (FA) stabilising agent in a polyvinyl alcohol (PVA) binder coating system.

Design/methodology/approach – The anthocyanin colorant was extracted using methanol acidified with 0.5% trifluoroacetic acid (TFA). FA was added to improve thermal stability of the colorant. The FA added colorant was mixed with PVA to develop a coating system. To test the ability of the coating mixture to withstand heat in the liquid state, spectroscopic studies were carried out in the visible region of the electromagnetic spectrum when the liquid samples had cooled down to room temperature after being heated at 80 and 90°C for 30 minutes. This procedure was repeated six times until a total heating time of 180 minutes has been accomplished. The liquid samples were also coated on glass slides, cured and then stored in different incubators at 30, 40 and 50°C. The visible spectrum was taken everyday for 30 days to study the effect of storage temperature. Spectroscopic results were analysed in terms of intensity rate percentage (IRP).

Findings – In the liquid state, the anthocyanin-PVA mixture without FA showed lower absorbance compared to the mixture containing FA after heating at 80 and 90°C. This shows that FA can enhance the intensity of absorbance of the liquid coating mixture. The mixtures containing FA show increase in absorbance with increase in heating time. The same results are obtained for the coating on glass substrate where FA containing coatings show increase in IRP with time for all storage temperatures. Coating with 1% FA content showed better enhancement and stability.

Research limitations/implications – The colour of the untreated samples quickly faded during heating and storage at different temperatures. In this study, the addition of 0.5% and 1% FA stabilised and enhanced the colour intensity at 30, 40 and 50°C. Further improvements may find the mixture suitable as paint or coating materials and as nail varnish.

Practical implications – The results indicate the possibility of applying the FA stabilised anthocyanin-PVA, colorant-binder composition in a coating system.

Originality/value – The use of anthocyanin from *M. Malabathricum* as a colourant in a coating system or nail varnish is original. Anthocyanin pigments are normally used as colorant in foods.

Keywords Coatings technology, Colour fastness, Plants, Anthocyanin, Melastoma malabathricum, Ferulic acid, Polyvinyl alcohol, Intensity rate percentage

Paper type Research paper

Introduction

Melastoma malabathricum is a shrub that belongs to the Melastomatacea family and it is locally known as "pokok senduduk". It has oblong leaves, purple flowers and deep purplish-blue fruits. Fruits of *M. malabathricum* are technically classified as berries. The seeds are orange in colour (Wong, 2008).

Fruit pulp of *M. malabathricum* contains anthocyanin (Janna *et al.*, 2006). Anthocyanins are natural, water-soluble and non-toxic compounds suitable for a wide range of applications. Anthocyanins have become well-known alternatives to synthetic dyes (Andersen and Jordheim, 2006; Espin *et al.*, 2000). However, anthocyanins are susceptible to colour deterioration during storage. This delays their potential for commercialisation (Cabrita *et al.*, 2000; Cai *et al.*, 1998; Mazza and Brouillard, 1990; Tsai *et al.*, 2002). According to Mazza and Brouillard (1990), the colour stability of anthocyanins depends on a combination of factors, such as the structure and concentration of the anthocyanin, pH, temperature, light and the presence of complexing agents such as phenols and metals. In the food industry, for example, the thermal impact during processing enhances the formation

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Colour analysis of potential natural colourant from *Ixora siamensis* and *Melastoma malabathricum*

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Anthocyanins are an important group of natural pigments that are responsible for many colours in plants. The variation in colour, depending on the pH, makes them a unique source of natural colourant. In this study, pigments from the fruits of *Ixora siamensis* and the fruit pulps of *Melastoma malabathricum* were extracted using trifluoroacetic acid–methanol solution. Spectral measurements (380–780 nm) were performed using visible spectroscopy with colour analysis software at different pHs (initial extracts were 1, 5, 7, 9 and 11). The colours of the solutions were expressed as colourimetric coordinates in the Commission Internationale de l'Eclairage (CIE) laboratory scale using L^* (lightness), C^* (chroma), $H°$ (hue angle notation h_{ab}), $a^*/-a^*$ (redness and greenness) and $b^*/-b^*$ (blueness and yellowness) for the D65/2°CIE Illuminant/Observer condition. In this work, the colour parameters were observed for natural colourant with and without blending with polyvinyl alcohol for both species (*Ixora siamensis* and *Melastoma malabathricum*). The relationships between the colour parameters (colourimetric indexes and CIELab variables) with pH variation and species dependence were discussed in this paper.

Keywords: Natural colourant, Anthocyanins, Colourimetric indexes, pH, Colour measurement, CIELab

Introduction

There has been much interest in the development of new natural colourants, which is apparently due to the strong consumer demand for more natural products, at least in some countries. The current consumer preference for naturally derived colourants is associated with their image of being healthy and of good quality. Natural colourants have become increasingly popular with consumers because synthetic colourants tend to be perceived as undesirable and harmful; some are considered to be responsible for allergenic and intolerance reactions.[1] According to Zhang *et al.*,[2] the development of new and alternative sources of natural colourants is worthwhile as the demand for natural colourants increases. Scientific research on the chemistry of colours, in the theoretical and applied level, is essential in order to improve the colourants from plants. The need to avoid the use of synthetic colourants and move towards the use of natural colours has also increased research during the past decades.

Anthocyanins are natural pigments that are widely distributed in nature. Anthocyanin colour molecules are subclasses of flavonoid. They are responsible for the red, purple and blue pigments in many flowers, fruits and vegetables. Anthocyanins are highly unstable and easily susceptible to the degradation process. The stability of anthocyanins is affected by pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins and the presence of other compounds, such as other flavonoids, proteins and minerals.[3] Anthocyanins belong to the flavonoid group of polyphenols. They have a $C_6C_3C_6$ skeleton typical of flavonoids. Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation, for example the flavylium cation in very acidic solutions.[4]

A solution of anthocyanin may exhibit different colours, depending on the pH of the solution.[5] As the pH increases, the anthocyanic nucleus is affected by important structural changes,[6] causing a dramatic loss of absorptivity in the visible region.[7,8] Below the pH 2 level, anthocyanins appear red due to the presence of flavylium cations, whereas at pH 6, the flavylium cation is converted into purple quinonoidal bases. Consequently, an adequate description of the colour variations in anthocyanins caused by co-pigmentation or pH requires the following:

(i) that the spectral variations considered should be those affecting the entire spectral curve, not only its visible λ_{max}
(ii) that the three cited colour attributes should be employed
(iii) that these should refer to one (or more) light source(s) and observer(s) condition(s).[9]

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Pigment & Resin Technology

Effects of UV-B irradiation on poly (vinyl alcohol) and Ixora siamensis anthocyanins-coated glass

Journal:	*Pigment & Resin Technology*
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Manuscript Type:	Original Article
Keywords:	Anthocyanins, Ixora siamensis, co-pigment, UV-B, glossiness, UV-Visible spectroscopy

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Body: @@date to be populated upon sending@@

Dear Professor Arof,

Manuscript ID PRT-12-2010-0114.R1 entitled "Effects of UV-B irradiation on poly (vinyl alcohol) and Ixora siamensis anthocyanins-coated glass" which you submitted to Pigment & Resin Technology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

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